The Manual

The
OXOID MANUAL
8th Edition 1998

Compiled by E. Y. Bridson
(former Technical Director of Oxoid)

Price: £10

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Printed in the United Kingdom.

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1
INTRODUCTION

November 1998

INTRODUCTION

The origins of OXOID Ltd go back to the beginnings of the science of microbiology.

Justus von Liebig (a famous chemist who clashed with Louis Pasteur about the microbiological cause of fermentation) had long been concerned about malnutrition in the poor of Europe. In 1860 he devised a concentrated meat extract which could be stored at room temperature without risk of spoilage. He called it ''Extractum carnis'' and he hoped it could be made available to everyone. This hope could not be achieved in Europe because of the high price of meat. However, in 1861, George Christian Giebert, a Belgian engineer working in Uruguay, read of this work and of Liebig's promise to help anyone who could produce the Extract to the same high standards. Both men knew that in South America, cattle were being slaughtered in thousands, solely for their hides and fat, the meat being abandoned to rot. Giebert visited Liebig in his Munich factory, learned the process and raised money in Antwerp to create a meat extract factory at Fray Bentos in Uruguay. Liebig approved of Giebert's product and allowed it to be called Liebig Extract of Meat. By 1865, production was so successful that the company was running out of money. This problem was solved when the Liebig Extract of Meat Company was formed and registered in London that same year. Both scientist and engineer had succeeded in their tasks. When Liebig died in 1873, he knew that his excellent extract was available to all in Europe. When Giebert died, a year later, he knew that he had established a sound industrial basis for the production of high quality products. Later more factories were established in South America, with surrounding ranches to breed cattle.

After Liebig's death, it was no longer possible to protect the great man's name on the bottle of Extract. Inferior Liebig Extracts began to appear on the market. To overcome this problem the Liebig Extract of Meat Company registered the trade mark LEMCO, from its initials.

Whilst sales of LEMCO and its by product Corned Beef continued to rise, the Company expanded its product range. Another meat extract, OXO was developed for English taste which preferred its high salt, low fat piquant flavour. It was this product which formed the penny OXO cube, a cheap and convenient form of nourishment. The commencement of the First World War in 1914 severed all links with Belgium and the Liebig marketing company Oxo Limited was formed in London that same year to sell products in the UK.

In 1924 Oxo Limited formed a Medical Division to sell glandular products to doctors under the trade name OXOID. About this time, LAB-LEMCO was developed for use in culture media. It was formulated from pale-coloured, low fat meat extracts which were more appropriate for the growth of micro-organisms. This was also the period when Liebig-Oxo increased investigation into enzymic- and acid-hydrolysis of meat and vegetable proteins to increase flavour and amino-nitrogen content of OXO. This work would eventually lead to the familiar peptones, such as Bacteriological Peptone L37.

The Second World War in 1939 presented new challenges and opportunities for change. The formation of the Emergency Pathology Service (EPS) to combat epidemics and the threat of biological warfare, meant that bacteriological investigations increased greatly. The development of penicillin in the 1940s gave further impetus to this activity. The EPS was transformed into the Public Health Laboratory Service and penicillin was followed by many other antibiotics. The Medical Division of Oxo Ltd., began to turn its attention to this rapidly growing market where infectious disease diagnosis and the industrial production of allied biologicals were of increasing importance. When, in 1957, it was decided to stop sales of pharmaceutical products, the replacement products (Oxoid Culture Media) were already being developed. Experience in the War had shown the value of dehydrated media and it was decided that this would be the main activity of the Oxoid Division. So successful was this decision that in 1965, Oxoid Limited was created as a separate subsidiary company of Liebig Extract of Meat Company.

In 1968 Liebig Extract of Meat Company merged with Brooke Bond Limited. The merged company was given the name Brooke-Bond Oxo and trade in culture media continued under Oxoid Limited.

In 1984 Brooke-Bond Oxo was purchased by Unilever Plc and for the first time in its history Oxoid was separated from Oxo. This prepared the way for all Unilever's medical products companies to be relaunched under a single international corporate identity, UNIPATH.

Finally, in 1996 Unilever decided to concentrate more on its core businesses and as a result Oxoid became an independent company for the first time in its history.

During the entire period of the Company's development outlined above the science of bacteriology was itself evolving with considerable speed. Early observers of microscopic life forms including Needham (1745) had recognised the need for the preparation of suitable nutrient fluids for their growth but there was a lack of uniformity in the methods followed.

The study of nutrient media possessing more exact composition was initiated by Pasteur in 1860. Cohn developed this work and published the formula for his ''normal bacterial liquid'' in 1870. Klebs noted Needham's early observations that saprophytic and putrefactive bacteria grew particularly well in a watery extract of meat and used a culture fluid of this nature when he commenced study of pathogenic bacteria in 1871. Nageli first described ''peptone'' in 1880. He has been credited as the first bacteriologist to discover that chemo-organotrophic organisms grow best in culture media containing a partially digested protein. Robert Koch quickly took up this development and in 1881 described his culture medium which was made from an aqueous meat extract to which was added peptone and sodium

chloride. To this day this simple formula is the basic one for culture media.

In the following year (1882) Heuppe described the labour saving convenience of substituting commercial meat extract for Koch's watery extract of fresh meat. By 1902 an American text book of bacteriology was recommending the use of LEMCO for this purpose. This is quite possibly the first record of exporting culture media ingredients by the company.

It will be seen that the business of manufacturing dehydrated culture media was a natural consequence of the converging commercial activities of Oxoid and the development of the science of microbiology. The early history explains why OXOID is one of the very few producers of culture media that actually manufactures its own extracts and hydrolysates.

The OXOID Quality Policy

It is the policy of OXOID, Basingstoke to manufacture and sell OXOID products which are fit for the purpose for which they are intended and are safe in use.

OXOID Ltd (Basingstoke) is registered with the BSI to BS EN ISO 9001 (Reg No. FM 09914) with extended scope to include BS EN 46001: 1997. This standard endorses our quality management system for products manufactured at the Basingstoke site and includes: Dehydrated Culture Media, Selective Supplements, Sterile Reagents, Biochemical Reagents, Laboratory Preparations, Signal Blood Culture System bottles, Susceptibility Discs in cartridges, Diagnostic Reagents, Salmonella Rapid Test and Listeria Rapid Test.

Ready Prepared Media and Lab Ready Media are manufactured by G. M. Procter and are covered under BS EN ISO 9002 Reg No. FM 27644.

The essential elements of the Oxoid Quality management System include:

- product lot testing according to a defined protocol
- documented complaints and technical enquiries procedure
- policy for raw material procurement
- good manufacturing practice combined with in-process control
- comprehensive training for staff at all levels
- conformance to statutory Health and Safety and Environmental requirements

The Oxoid Quality Policy functions through all procedures described above and maintains the company's high reputation for the performance of its products.

Storage of OXOID Microbiological Products

To ensure optimum performance from OXOID products they must be stored under specified conditions and for no longer than the allocated shelf-life. The storage conditions and expiry date of each product are shown on the label, container or product insert. Products should be used in order of their lot/batch numbers.

Light
All prepared culture media should be stored away from light and exposure to direct sunlight avoided at all times.

Humidity
Sealed glass and plastic containers are unaffected by normal laboratory humidity. Opened containers of dehydrated powders will be affected by high humidity. Hot, steamy media preparation rooms are unsuitable environments to store containers of culture media; particularly containers which are frequently opened and closed. An adjacent cooler room or an adequate storage cupboard are preferable storage areas.

Temperature and time
The temperature storage conditions of culture media and their components vary widely. The following product groupings will help to differentiate the various requirements.

Prepared Agar and Broth Media (PM, R products)
Store at 2–8°C. do not allow the products to freeze.
Shelf life 3 months to 2 years.

Biochemical Reagents (BR products)
Store at 2–8°C.
Shelf life 1 to 5 years.

Gas Generating Kits
Store at 2–25°C. in a dry place. Do not store these kits at a higher temperature for long periods.
Shelf life 3 years.

20 months	20 months	20 months
Anaerogen™	Campygen™	CO_2Gen™

Selective and Sterile Reagents (SR products, Selective supplements)
Store at 2–8°C.

except
Horse Serum SR35
store at –20 to +8°C.
Nitrocefin SR112
Reconstitution fluid SR112A
store at –20 to +8°C.
Penase SR129
store at –20°C.
Shelf life 1 week to 2 years.

Culti Loops
Store at 2–8°C. or frozen for *Campylobacter* sp.
Shelf life 6–10 months (except *Campylobacter jejuni* – 4–6 months).

Toxin Detection Kits
Store at 2–8°C.
Shelf life 1 year.

Salmonella Rapid Test
Store at room temperature 15–25°C.
Shelf life 1 year to 15 months.

Listeria Rapid Test
Store at 2–8°C.
Shelf life maximum 18 months.

Dip Slides
Store at 10–15°C.
Shelf life 6–9 months.

Quanticult
Store at 2–8°C.
Shelf life 6–10 months.

Diagnostic Reagents (DR products)
Store at 2–8°C, do not freeze.
Shelf life 9 months to 2 years.

Diagnostic Discs (DD range)
Store at –20°C but keep working stock at 2–8°C.
Shelf life 1 to 2 years.

DRYSPOT
Store at room temperature 15–25°C.
Shelf life 2 years.

Susceptibility Discs
Store at –20°C but keep working stock at 2–8°C.
Shelf life 1 to 3 years.

Dehydrated Culture Media (CM, L products)
Sealed, unopened containers should be stored at room temperature 15–20°C.

Opened containers should have the cap carefully and securely replaced. It is important that opened containers are stored in a dry atmosphere at room temperature.

Shelf life 1 to 5 years.

Prepared Plates Of Culture Media
Poured plates of agar media are especially vulnerable to infection, dehydration and chemical degradation. Aseptic preparation and storage are essential to protect plates from microbial infection.

Water losses on storage can be minimised by impermeable wrapping and/or storage at 2–8°C.

Chemical degradation e.g. oxidation or antimicrobial loss, can be retarded by protection from light, heat and dehydration.

Therefore storage of prepared plates at 2–8°C (unless otherwise stated) in the absence of light and protected against moisture loss will minimise agar media deterioration from these defects.

It is important, however, to monitor the storage of prepared plates by quality control tests so that any deterioration can be detected and the storage period accurately determined. Simple weighing tests of fresh and stored plates will determine the rate of moisture loss. Greater than 5% loss of weight will indicate a significant loss of water.

PRECAUTIONS IN MICROBIOLOGY

Manipulations with micro-organisms may release some of them into the environment and lead to laboratory-acquired infections. Such release may be entirely accidental or it may be intrinsic in the technique or equipment used. Even the most careful worker, using the best methods and the correct equipment, is not immune from accidents and errors. Over 4500 such infections have been reported so far this century[1].

Accidents that release micro-organisms include spillage and breakage. Activities that frequently release micro-organisms include opening cultures, using inoculating needles and loops, using hypodermic needles, pipetting, mixing, homogenising, and centrifuging[1].

Micro-organisms released into the environment may enter the bodies of workers and other people in and around the laboratory and initiate infections. Those most at risk are clinical laboratory and research staff. Even in industry, e.g. in food testing laboratories, pathogens that are present in small numbers in samples submitted for examination may be concentrated by culture into infectious doses.

ROUTES OF INFECTION

Micro-organisms may enter the human body by any of several routes: through the respiratory tract, the alimentary tract, the skin, and the conjunctivae.

The Respiratory Tract – Inhalation
Very small droplets of liquids – aerosols – that may contain micro-organisms are generated when films of liquids are broken, e.g. when cultures are opened or broken, liquids are pipetted violently, bursting bubbles, splashes, falling drops impacting on surfaces, and breakages in centrifuges. The smallest of these droplets, those less than 5μm in size, remain suspended in the air and dry rapidly. The organisms they contain then become ''droplet nuclei'' and are moved around the room or to other parts of the building by air currents. If they are inhaled they are small enough to reach the alveoli, where they may initiate an infection. Larger droplets sediment rapidly under the influence of gravity and may contaminate benches, equipment or the hands. If they are inhaled they are trapped and removed in the upper air passages.

The Alimentary Tract – Ingestion
Workers' hands may be contaminated by spillage and by the larger aerosol droplets. The organisms may then be transferred to the mouth by the fingers, or by contaminated pencils, pipettes, food etc.

The Skin
Although the intact skin is a good barrier against micro-organisms, the exposed parts, e.g. the hands and face, are frequently damaged by small cuts and abrasions, many of which may not be visible to the naked eye. These are portals of entry for micro-organisms.

In addition, ''sharps'' injuries are not uncommon in laboratories[2]. Pricks and cuts with needles, knives, broken glass, etc. will allow the entry of micro-organisms.

The Conjunctivae
The very thin membranes surrounding the eyes are readily penetrated by micro-organisms in splashes or from contaminated fingers. Some people touch their eyes several times an hour.

CLASSIFICATION OF MICRO-ORGANISMS ON THE BASIS OF HAZARD

It is obvious that not all micro-organisms have the same capacity to cause infections, and that infections vary in their incidence, their severity, and the availability of prophylaxis and therapy. By international agreement micro-organisms are now classified into groups or classes according to the hazard they offer to workers and the community. There are four groups, ranging from the relatively harmless to the very hazardous. The wording varies slightly from state to state and that used in Europe[3] is shown in Table 1.

Lists of bacteria, viruses, fungi and parasites in Groups 2, 3 and 4 have been published by various national and international agencies, e.g. the European Union[3,4]. Micro-organisms not listed in these Groups are assumed to be in Group 1, although some of them may be responsible for allergies. There are inevitable disagreements, globally, because of differences in the

TABLE 1

Classification of micro-organisms on the basis of hazard and laboratory containment level

Class	*Description*	*Laboratory*
1	Unlikely to cause human disease.	Level 1
2	May cause human disease; might be a hazard to laboratory workers; unlikely to spread in the community; laboratory exposure rarely causes infections; effective prophylaxis and therapy available.	Level 2
3	May cause serious human disease; may be serious hazard to laboratory workers; may spread in the community; effective prophylaxis and therapy available.	Level 3
4	Causes severe human disease; serious threat to laboratory workers; high risk of spread in the community; no effective prophylaxis and therapy.	Level 4

Based on the classification of the UK Advisory Committee on Dangerous Pathogens[6].
Classes (also known as Groups) 2, 3 and 4 include known pathogens.
Class 4 contains only viruses.

geographical distribution, incidence, and local significance[5].

CLASSIFICATION OF LABORATORIES ACCORDING TO HAZARD GROUP

It follows from the classification of micro-organisms on the basis of hazard that precautions against laboratory-acquired infections should vary from minimal for those in Group 1 to maximum security for those in Group 4. Such precautions and safety requirements have been codified as Containment of Biosafety Levels[1,3–5]. These are outlined in Table 2. General precautions are considered below.

Where there are disagreements in classifications the local system should be regarded as the minimum, but there is no reason why microbiologists, if they think fit, should not use higher levels of precautions than those prescribed nationally.

GENERAL PRECAUTIONS AGAINST LABORATORY-ACQUIRED INFECTIONS

There are several international and national guidelines and codes of practice[1,5–10]. Only outlines can be given here.

Personal Protection
Protective clothing should be worn at all times in the laboratory. Gowns, coats and overalls should be fastened at the sides or back, cover the chest and neck areas and fit closely at the wrists. Workers should remove this clothing before leaving the laboratory and not wear it in rest rooms, offices, libraries etc. Gloves should be worn if there is a risk of contaminating the hands, especially with blood. Disposable (latex) gloves should be worn once only and then autoclaved with other laboratory wastes. Re-usable gloves should be washed while still on the hands and then disinfected before re-use[5]. Safety spectacles should be worn during microbiological and chemical manipulations. Hands should be washed often and always before leaving the laboratory.

Laboratory Equipment
Inoculating Loops
Long wires vibrate and shed droplets, as do large and poorly made loops. The wires should be no longer than 6cm and the loops not more than 2mm in diameter and completely closed. Plastic disposable loops are to be preferred as they do not need flaming but may be placed in disinfectant immediately after use.

Glassware
Chipped and scratched glassware is hazardous and should never be used.

Pasteur Pipettes
Glass Pasteur pipettes should not be used as they are often responsible for cuts and punctures of the skin. Soft plastic pipettes are safer.

Hypodermic Needles
To avoid ''needlestick'' accidents pipettes and cannulas should be used instead of hypodermic needles. Opening devices for vaccine and septum-capped bottles are available.

Centrifuges
Accidents with centrifuges may release massive aerosols. They are often the result of improper handling. Centrifuges should be placed on low benches so that all operators can see the inside of the bowl when loading them. Buckets and trunnions should be inspected regularly for evidence of corrosion and hairline cracks; any suspect parts should be discarded. When not in use buckets should be placed upside down in racks to drain any fluid used in balancing. Buckets should be paired by weight and labelled accordingly. Paired buckets should be placed opposite one another for use. At least 2cm clear space should be left between the top of the fluid in a centrifuge tube and its rim. Centrifuge tubes should be stoppered and sealed buckets used for any material that is potentially infectious. Paired buckets, with tubes *in situ*, should be balanced by adding 70% alcohol (NOT saline, which may corrode

TABLE 2

Summary of laboratory design features for laboratory containment levels

	Containment Level			
	1	***2***	***3***	***4***
Laboratory isolated and sealable for decontamination	–	–	+	+
Directional ventilation (inward)	–	D	+	+
Filtered air exhaust	–	–	+	+
Double door entry	–	–	O	+
Airlock with shower	–	–	–	+
Autoclave on site	+	+	+	+
in workroom	–	–	O	+
double ended	–	–	–	+
Microbiological safety cabinets				
Class I or II available	–	+	+	+
in workroom	–	–	+	+
Class III	–	–	O	+

Based on WHO Laboratory Biosafety Manual[5]
Key: – not required + essential D desirable O optional

metal, leading to mechanical failure) to the space between the tube and the bucket. Instructions for use of centrifuges and action to be taken if a centrifuge tube breaks, usually indicated by a sudden change in sound and/or visible imbalance of the machine, should be posted adjacent to each machine.

Physical hazards associated with centrifuges are discussed in detail by Kennedy[11].

Water Baths
The water in water baths may become contaminated from the outsides of culture tubes or the leakage of their contents. These baths, even those operated at temperatures >60°C should be emptied when not in use or a deposit may form in which micro-organisms can grow. A disinfectant that does not attack metals may be added to the water in baths that are in continuous use (hypochlorites should not be used; see below).

Homogenisers and Shakers
Bench-mounted models may generate aerosols and should be covered, (e.g. by clear plastic boxes) when in use. These covers should be disinfected after use. Hand-held homogenisers should be held in a wad of cotton wool in case they break. Homogenisers and containers from shakers should be opened in microbiological safety cabinets.

Pipetting
Pipetting by mouth, even water, should be banned. Pipetting devices should be provided. Pipettes should not be blown out vigorously, otherwise bubbles and aerosols may be formed.

Microbiological Safety Cabinets
These should conform to national standards and should be tested regularly by independent engineers to ensure that their performance is in accordance with the requirements of that standard. These cabinets are designed to protect the user from the inhalation of infectious aerosols and air-borne particles. They give no protection against spillages of cultures or against chemicals. Class II and Class III cabinets also protect the test or product from external air-borne contamination.

Microbiological safety cabinets should be used only by experienced personnel who have received proper instructions about their limitations. They must not be used as fume cupboards or for work with flammable or toxic substances.

They should be decontaminated at regular intervals by qualified staff who follow manufacturers', or other recognised procedures[1,5,10].

Laminar Outflow (clean air) Cabinets
These are NOT microbiological safety cabinets. They are designed to protect the work from external air-borne contamination and do not protect the worker, whose face and respiratory tract receive air that has passed over the workpiece. (See Cell and Tissue culture, below).

Fume Cupboards
Fume cupboards are designed to protect workers and the environment from toxic chemical fumes and gases. They should not be used for micro-organisms or other living material.

SPILLAGE AND BREAKAGE

Spillage of cultures and chemicals and breakage of vessels containing them must be reported immediately to the supervisor or local safety office. If the spillage is considerable the room should be vacated pending decontamination by qualified staff (see below).

Instructions for dealing with small-scale spillages and breakages should be posted in each laboratory, and should include the following:

- wear heavy-duty gloves
- cover the spillage/breakage with absorbent material, e.g. large paper towels
- pour disinfectant (see Table 3) over the paper towels and leave for at least 15 minutes
- scoop up the paper towels with a dust pan or stiff cardboard and place them along with the dust pan or cardboard, along with any broken glass into a laboratory discard container
- pick up any residual broken glass with forceps and add it to the discard container
- cover the area again with paper towels and pour on more disinfectant. Leave for 30 minutes before any further cleaning up
- autoclave the discard container.

PRECAUTIONS AGAINST BLOOD-BORNE INFECTIONS

In addition to the precautions listed above personnel who handle blood specimens or blood-stained material should wear high quality disposable gloves and also plastic disposable aprons over their normal protective clothing. Guidelines for the safe handling in laboratories of materials that may contain hepatitis and/or the human immunodeficiency virus have been published[1,7,8,12,13].

PRECAUTIONS WITH CELL AND TISSUE CULTURE

Separate accommodations should be provided to minimise contamination of cultures.

Some cells and tissue cultures may contain adventitious and unidentified micro-organisms or viruses from which the operator must be protected. All work with cells and cell lines should therefore be conducted in Class II microbiological safety cabinets. Laminar outflow cabinets (see above) must NOT be used.

STERILISATION, DISINFECTION AND DECONTAMINATION

These terms are not interchangeable. In microbiology:

Sterilisation – implies the complete destruction of all micro-organisms.

Disinfection – is the destruction or inactivation, usually by chemicals, of the vegetative forms of micro-organisms and the spores of some of them. Not all spores are inactivated. Not all spores are inactivated by chemical disinfectants.

Decontamination – usually means making equipment, materials and waste free from infectious agents.

Sterilisation
Here, this is restricted to autoclaving. For other methods, e.g. hot air, standard textbooks should be consulted[1,10].

The hazard most frequently encountered in autoclaving is failure to sterilise, i.e. to achieve and maintain the temperature/time ratio that is known to kill micro-organisms. (The physical hazards of autoclaving are described elsewhere[11]).

Autoclaves should be used only by personnel specifically trained and employed for that purpose. Infected materials and ''clean'' articles should be treated in separate loads and preferably separate autoclaves. Autoclaves should not be tightly packed: space must be left between articles in the load to enable steam to circulate freely.

The ''Holding time at temperature'' (HTAT) for steam sterilisation is normally 20 minutes at 121°C. The time begins when the temperature **in the load** has reached 121°C as indicated by the recorder of the thermocouple in that load, NOT when the drain temperature reaches that temperature[1,10].

Higher temperatures are required for the treatment of material containing ''unconventional agents'' (e.g. scrapie, CJD, etc.).

Control of Sterilisation
In modern autoclaves this is achieved by instrumentation (thermocouple probes and recorders). It is advisable, however, to include some form of indicator, e.g. ''autoclave tape'' in each load, and to check the HTAT independently at regular intervals. Alternatively, or in addition, biological tests may be used in the form of strips that contain *Bacillus stearothermophilus*[1,10].

Chemical Disinfection
Disinfectants vary in the action against bacteria, spores, fungi and viruses and should be chosen in accordance with the intended use. Most disinfectants are toxic, in varying degrees, and precautions, e.g. eye protection, should be taken when stock solutions are diluted.

Table 3 summarises the properties of some commonly used chemical disinfectants.

Disinfectants should be diluted according to the manufacturers' instructions. It is best to prepare dilutions daily as some deteriorate if use-dilutions are stored. For most purposes hypochlorites are adequate and should be diluted to contain 1,000–2,500 ppm available chlorine for normal work and 10,000 ppm for blood and high concentrations of protein. Industrial hypochlorite solutions usually contain 100,000 ppm available chlorine and should be diluted 1–2.5% or 10%.

Bench discard jars and containers
A discard jar containing an appropriate disinfectant should be provided at every work station to receive small items such as slides, Pasteur pipettes and plastic loops. Large jars, for pipettes are also needed. Plastic containers are safer than glass. Articles placed in these containers should be completely submerged in the disinfectant. Discard containers should be emptied and replaced daily.

Containers for discarded cultures should also be provided at each work station. These should not leak, be shallow – not more than 25cm deep to facilitate steam penetration during autoclaving, and preferably of heat-resistant plastic. Plastic bags, usually blue or transparent with blue lettering, are used in some (mostly UK) laboratories. They should be supported in the containers described above.

Decontamination of Benches, Equipment and Rooms
Benches should be wiped down with a suitable disinfectant at the end of the working day (gloves should be worn). The accessible parts of equipment may similarly be disinfected but not with hypochlorites as they may attack metals.

TABLE 3

Properties of some disinfectants

	Active against							Inactivated by					Toxicity		
	Fungi	Bacteria		Myco-	Spores	Viruses		Protein	Materials		Hard	Deter-	Skin	Eyes	Lungs
		G+	G–	bacteria		Lipid	Non lipid		Natural	Man-made	water	gent			
Phenolics	+++	+++	+++	++	–	+	v	+	++	++	+	C	+	+	–
Hypochlorites	+	+++	+++	++	++	+	+	+++	+	+	+	C	+	+	+
Alcohols	–	+++	+++	+++	–	+	v	+	+	+	+	–	–	+	–
Formaldehyde	+++	+++	+++	+++	+++a	+	+	+	+	+	+	–	+	+	+
Glutaraldehyde	+++	+++	+++	+++	+++b	+	+	NA	+	+	+	–	+	+	+
Iodophors	+++	+++	+++	+++	+	+	+	+++	+	+	+	A	+	+	–
QAC	+	+++	++	–	–	–	–	+++	+++	+++	+++	A(C)	+	+	–

+++ Good: ++ Fair: + Slight: – Nil: V Depends on virus: a Above 40°C: b Above 20°C: C Catonic: A Anionic

From Collins, C.H. (1993) *Laboratory Acquired Infections. 3rd.edn.* by permission of the publishers Butterworth-Heinemann, Oxford

Equipment to be serviced must also be decontaminated in this way and clearly labelled to indicate that this has been done and that it should not be used until after servicing.

The working surfaces and inner walls of microbiological safety cabinets should be swabbed with a suitable disinfectant, and the cabinets be fumigated with formaldehyde, as indicated above before filters are changed or maintenance carried out.

Rooms rarely need disinfection unless a major accident has released massive aerosols. Formerly this was done by formaldehyde fumigation, but this is now regarded as hazardous and uncertain. Spraying or washing with disinfectant/detergent mixtures is safer and more effective.

DISPOSAL OF INFECTED WASTE

Infected laboratory waste is included in the definitions of clinical waste and must ultimately be incinerated. Table 4 lists the materials that should be regarded as infectious in microbiological and clinical laboratories. As these are likely to be the most heavily infected of all such waste and may have to travel along the public highway, often for long distances. It is prudent to autoclave it first[1,14,15].

CONCLUSIONS

Every microbiological laboratory should have written safety policy and instructions that describe in full the safety precautions deemed necessary by the Director and Safety Officer.

All members of the staff should be aware of the authorised procedures for containing and destroying micro-organisms.

A schedule of regular microbiological safety cleaning should be maintained for all working surfaces and adjacent areas.

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TABLE 4

Infected and potentially infected waste from microbiological laboratories

Disposables other than sharps

- Specimens or their remains (in their containers) submitted for tests containing blood, faeces, sputum, urine, secretions, exudates, transudates, other normal or morbid fluids but not tissues.
- All cultures made from these specimens, directly or indirectly.
- All other stocks of micro-organisms that are no longer required.
- Used diagnostic kits (which may contain glass, plastics, chemicals and biologicals).
- Used disposable transfer loops, rods, plastic Pasteur pipettes.
- Disposable cuvettes and containers used in chemical analyses.
- Biologicals, standards and quality control materials.
- Food samples submitted for examination in outbreaks of food poisoning.
- Paper towels and tissues used to wipe benches and equipment and to dry hands.
- Disposable gloves and gowns.

Sharps

- Hypodermic needles (syringes attached if custom so requires).
- Disposable knives, scalpels, blades, scissors, forceps, probes.
- Glass Pasteur pipettes; slides and cover glasses.
- Broken glass, ampoules and vials.

Tissues and animal carcasses

Bedding from animal cages

Adapted from Collins and Kennedy[14] by permission of the authors and publisher.

2
CULTURE MEDIA

November 1998

CULTURE MEDIA QUALITY ASSURANCE

All manufacturing operations are conducted according to protocols which describe such procedures as the monitoring, maintenance, cleaning and calibration of equipment; plant sanitation; warehouse control of in-coming materials and materials under test; labelling control and handling, storage and distribution of finished goods. The master formula and accompanying documents for each lot/batch of product includes manufacturing control and packaging information pertaining to the product.

Quality tests on raw materials include identity tests, tests for performance and compatibility with other ingredients in a pre-production laboratory mix of the medium components. Additional tests are performed where required. For example, peptones are examined physically, chemically and microbiologically. Agars are tested for clarity, gel strength, diffusion characteristics etc.

Dehydrated media mixtures are examined for appearance, homogeneity and moisture content. Representative samples are reconstituted and examined for colour, clarity, pH, gel strength (if agar is present), compatibility with post-sterilisation additives and for microbiological performance. The medium is challenged with small inocula of well-defined cultures to measure recovery of growth, colony size and morphology, colour reactions, differentiation and selectivity.

Special procedures such as antimicrobial susceptibility tests are performed where appropriate for the recommended use of the medium.

All tests are performed in parallel with a previously approved reference batch of the medium. This use of a control standard medium with each test ensures uniformity in reading the results.

Samples of each manufactured lot/batch are retained for the total shelf-life of the product. Stability tests and lot-to-lot uniformity tests are carried out using these retained samples.

FORMULATION OF CULTURE MEDIA: DEVELOPMENT AND MANUFACTURE

The formulation of all Oxoid culture media are published in Section 2.7 and the components can be divided into different roles or functions:

1 **Nutrients:** proteins/peptides/amino-acids.

2 **Energy:** carbohydrates.

3 **Essential metals and minerals:** calcium, magnesium, iron, trace metals: phosphates, sulphates etc.

4 **Buffering agents:** phosphates, acetates etc.

5 **Indicators for pH change:** phenol red, bromo-cresol purple etc.

6 **Selective agents:** chemicals, antimicrobial agents.

7 **Gelling agent:** usually agar.

There is often an overlap of functions of some media components, thus protein hydrolysates will supply amino-nitrogen, energy, some metals/minerals and act as buffering agents. Phosphate buffers are important suppliers of minerals and agar contributes metals.

1 Nutrients

Naegeli is credited with the earliest publications (1880/82) describing the requirements of micro-organisms for a protein component which he called 'peptone'.

Later work showed that the group of bacteria, now defined as chemo-organotrophs, required amino-nitrogen compounds as essential growth factors in their culture media.

Meat infusions contain water-soluble fractions of protein (amino-acids and small peptides) along with other water-soluble products such as vitamins, trace metals, minerals and carbohydrates (glycogen). Such infusions or extracts may have been regarded as 'peptones' but their amino-nitrogen content was usually too low to sustain the growth of large numbers of bacteria.

It was not until deliberate attempts were made to hydrolyse proteins with acids or enzymes that sufficiently high concentrations of water-soluble protein fractions (peptides) were made available for bacterial growth. Many nutrient media usually contain a mixture of protein hydrolysate (peptone) and meat infusion (meat extract/Lab-Lemco).

The difficulties associated with the production of protein hydrolysates were soon recognised and commercial suppliers of peptones became established by the 1920s. The commercial supply of dried peptone eventually led to complete culture media being produced in the form of dehydrated media.

Although meat was the first and most obvious protein to hydrolyse, other proteins were tried later and some showed specific advantages which ensured their retention in culture media to this day. Casein hydrolysate with its pale colour and high tryptophan

content and soya peptone with its high energy carbohydrate content are popular examples of non-meat peptones.

A detailed description of these products is given in Section 3.1 'Peptones-Hydrolysates'.

The nutrient components of culture media are carefully selected to recover the required spectrum of organisms in the sample e.g. coliforms or anaerobes. General purpose media such as blood agar in its various forms will often contain mixtures of peptones to ensure that peptides of sufficient variety are available for the great majority of organisms likely to be present. However, more demanding organisms will require supplemental growth factors to be added and examples of such requirements can be seen in media for *Legionella* species.

Most of the components used for the nutrition of micro-organisms are undefined and require extensive testing with careful selection to ensure a reasonable degree of uniformity. Would it not be better to use wholly defined peptides and amino-acids to produce a totally defined medium? Whilst such media would improve uniformity, experience has shown that they lack good performance as general purpose media. They would also be very expensive compared with undefined media. The use of totally defined culture media is an understandable goal of most microbiologists but defined media have yet to prove themselves equal in performance to currently used complex mixtures of meat and plant protein hydrolysates.

2 Energy
The most common substance added to culture media as a source of energy to increase the rate of growth of organisms is glucose. Other carbohydrates may be used as required.

Carbohydrates added to media at 5–10 grammes per litre are usually present as biochemical substrates to detect the production of specific enzymes in the identification of organisms. It is usual to add pH indicators to such formulations.

3 Essential Metals and Minerals
The inorganic essential components of culture media are many and can be divided on a semi-quantitative basis:

Typical macro-components (gm/litre):
Na, K, Cl , P, S, Ca, Mg, Fe.

Typical micro-components (mgm-microgm/litre):
Zn, Mn, Br, B, Cu, Co, Mo, V, Sr, etc.

As previously mentioned, a formulation may not have specific metals and minerals listed in its formulation. In such cases it is assumed that all the factors required are present in the hydrolysates, buffers and agar components.

4 Buffering Agents
It is important that the pH of a culture medium is poised around the optimum necessary for growth of the desired micro-organisms. The use of buffer compounds at specific pK values is especially necessary when fermentable carbohydrates are added as energy sources.

Phosphates, acetates, citrates, zwitterion compounds and specific amino-acids are examples of buffering agents that may be added to culture media.

A side effect of such compounds is their ability to chelate (or bind) divalent cations (Ca++ and Mg++). Polyphosphate salts, sometimes present in sodium phosphate, are compounds which can bind essential cations so firmly that they are made inaccessible to the micro-organisms.

The effect of these binding or chelating agents will be seen in diminished growth or failure to grow at all, unless care has been taken to supplement the essential cations in the formulation. Opacity forming in a medium, after heating or on standing at 50°C for several hours, is commonly caused by phosphate interaction with metals. Such phosphate precipitates can very effectively bind Fe and lower the available amount of this essential metal in the medium.

5 Indicator Substances
The addition of coloured indicator substances is a very effective way of detecting fermentation of specific carbohydrates in a culture medium. Such compounds should change colour distinctly and rapidly at critical pH values.

Most of the compounds used e.g. phenol red, bromo-cresol purple, fuchsin, etc., are toxic and it is essential to use low concentrations of pre-screened batches/ lots. Known sensitive strains of micro-organisms are used in the screening tests.

6 Selective Agents
Chemicals or antimicrobials are added to culture media to make them selective for certain micro-organisms. The selective agents are chosen and added at specific concentrations to suppress the growth of unwanted organisms in a polymicrobial sample. It is, of course, essential to have established that the selective agents, at the appropriate concentration, will allow uninhibited growth of the desired organisms.

Common chemical selective agents are: bile salts, dye-stuffs, selenite, tetrathionate, tellurite and azide. Antimicrobial agents are commonly used in mixtures when suppressing polymicrobial contaminating flora. Antimicrobials are more specific in their selective action than the chemical agents shown above. However, the critical weighing and heat-lability of most antimicrobials demand special care and post-sterilisation addition.

The wide variety of organisms and their almost infinite ability to adapt to changing conditions makes a truly selective medium unlikely. Selective media can be said to suppress most of the unwanted organisms and allow most of the desired organisms to grow. The final formulation is usually a compromise which achieves the best of these criteria.

7 Gelling Agents
Although gelatin is still used for a few specific media and carrageenans, alginates, silica gel and polyacrylamides are sometimes used as gelling agents, the outstanding gel-forming substance used in culture media is agar.

Hesse, a worker in Robert Koch's laboratory, is credited with its first use in culture media, although Frau Hesse gave him the idea from its use in table-jellies in hot climates.

Its inertness to microbial action, the unique setting and melting temperatures (38°C and 84°C respectively) the high gel strength which allows low concentrations of agar to be used, its clarity and low toxicity have contributed to its wide popularity with microbiologists. Its ability to retain its gel structure at 60°C makes agar of special value to culture media which have to be incubated at this temperature to isolate thermophilic organisms.

Agar is obtained from agarophyte sea-weeds mainly *Gelidium, Gracilaria* and *Pterocladia* species. It is extracted as an aqueous solution at greater than 100°C, decolourised, filtered, dried and milled to a powder.

Agar is not an inert gelling agent; it contributes nutrients and/or toxic agents to culture media, depending on the chemical processing carried out by the suppliers.

Microbiological agar is specially processed to yield a low toxicity, high clarity, low mineral and high diffusion gel.

Other Components
There are many other substances added to culture media for specific purposes e.g. growth factors for fastidious organisms, eH-reducing compounds for anaerobic organisms (thioglycollate and cysteine), whole blood to detect haemolytic enzymes and encourage the growth of organisms which are vulnerable to oxidation products.

Development and Manufacture of Culture Media
The development of dehydrated culture media is a process leading to the large-scale manufacture of a reproducible, stable product. The initial development of the formulation is usually carried out by microbiologists who wish to create a novel medium with specific characteristics or who wish to improve the performance of an existing product. Such work is usually written up in microbiological journals, having first been judged by some form of peer review and proved to be of special value by other workers in the field.

Simple conversion of the published formula into a mixture of dehydrated components is seldom achieved. Usually the peptone/hydrolysate base has to be adapted and variations in concentration of other components may be required. Laboratory mixes of the medium are prepared as R&D trials and after testing in the laboratory are sent to the originator for comment. Opportunity may also be taken to get the views of other experts in this field. Special strains of organisms may be required to check the finer points of performance.

Subject to good report, a trial batch will be manufactured and this will be used for larger trials and wider-scale testing. During these trials QC testing and performance criteria will be established and the specifications of the components will be determined. Bought-in components will have buying specifications and in-house components will have manufacturing specifications and standard-operating-processes produced. Stability trials will begin if there is confidence that the final formulation has been achieved.

The reports on the larger and wider-spread trials are studied and if the results are satisfactory preparation will be made to manufacture a full production batch/lot. All the components of the medium, including special protein hydrolysates which may have to be specially manufactured, are assembled and a laboratory mix tested to see that it meets the performance specification. Finally the components are milled, mixed and blended to produce a finely divided, homogeneous powder which is held in large containers for further testing before release.

All this work, plus literature, labels and product inserts is carried out under the aegis of R&D/Marketing. Subsequent production lots are manufactured under surveillance which includes GMP monitoring and end-product testing by the Quality Department.

No product can be released without clearance from The Quality Department.

GENERAL GUIDE TO THE USE OF OXOID CULTURE MEDIA

PREPARATION OF DEHYDRATED MEDIA

Dehydrated media are hygroscopic and are sensitive to moisture, heat and light. They are adversely affected by drastic changes in temperature e.g. hot/cold cycling temperatures which may occur between day and night laboratory temperatures in winter.

Storage conditions are usually indicated on the product label and should be followed.

1 Write on the label the date of receipt in the laboratory.

2 Store as directed on the label; usually below 25°C in a dry area, away from direct sunlight, autoclaves, drying ovens or other heat sources. Where indicated store at 2–8°C.

3 Check expiry date on the label, some media have significantly shorter shelf-lives than others.

4 Use stock in lot/batch number order. Do not open a new bottle until the previous bottle has been emptied. Note on the label the date the container is first opened. After use, make sure the container is tightly closed and return it to the designated storage area.

5 Order the medium in an appropriate size of container and in a quantity which accords to normal use requirements. A medium in a large container which has been opened many times will deteriorate on storage. Discard the medium if the powder is not free flowing, if the colour has changed or if it appears abnormal in any way.

RECONSTITUTION OF DEHYDRATED MEDIA

Complete instructions for the preparation of culture media are given on the label of each bottle. As a general rule it is wise to prepare one week's requirement only.

1 Use water prepared by distillation, deionisation or reverse osmosis. Toxic metal ions such as copper must be absent. Check the pH of the water, if below 5.5, heat to drive off CO_2 and re-check. The conductivity of the water should ideally be below 15 micro siemens (μS). Rinse glassware before use.

2 Prepare the medium in a vessel about twice the final volume of the medium to allow adequate mixing. Follow the instructions given on the label of each product.

3 Open the culture medium container away from draughts and moisture. Avoid inhaling the powder and prolonged skin contact. Weigh the powder quickly, accurately and without creating 'clouds of dust'. Reclose the container as soon as possible.

4 Pour half the required volume of water in the vessel, then the weighed quantity of medium and agitate briskly for a few minutes. Pour the rest of the water down the sides of the vessel to wash any adherent medium back into solution. This is an important step because dry culture media powder above the level of the water may not be sterilised in the autoclave and may be a source of contamination.

Agar-free media will usually dissolve with gentle agitation.

Media containing agar should be heated to dissolve the agar before autoclaving. Bring the medium to the boil without scorching or burning. Those media which should not be autoclaved will be ready to pour into dishes or other containers after this amount of heating. Most culture media will require final sterilisation in an autoclave at 121°C for 15 minutes.

The pH of the dehydrated medium has been adjusted so that the final pH of the prepared medium conforms with the label specification when the medium has been cooled to 25°C. Do not adjust the pH before sterilisation.

STERILISATION OF CULTURE MEDIA

Although sterilisation of culture media is best carried out in a steam autoclave at temperatures around 121°C it has to be recognised that damage is caused to the medium by the heating process.

Heat-treatment of complex culture media which contain peptides, sugars, minerals and metals results in nutrient destruction, either by direct thermal degradation or by reaction between the medium components. Toxic products caused by chemo-oxidation can also be formed during heat-treatment.

It is important, therefore, to optimise the heating process so that a medium is sterile after heating but minimal damage is caused to the ingredients of the medium. As a general rule it is accepted that short-duration, high-temperature processes are more lethal to organisms and less chemically damaging than are longer, lower temperature processes.

A general instruction for sterilising culture media in volumes up to one litre at 121°C for 15 minutes is given on each label. Autoclaves vary in performance, however, and thermocouple tests using different volumes of media should be carried out to determine the 'heat-up' and 'cool-down' times. It will be essential to do this when volumes of media greater than two litres are prepared. In order to avoid overheating large volume units of media, the 'heat-up' and 'cool-down' periods are normally integrated into the 121°C holding time.

Sterilisation Cycle
The sterilisation cycle can be divided into its four stages:

1 Chamber heat-up time
2 Heat penetration time of the medium container
3 Holding time at the prescribed temperature
4 Cool-down time for the chamber to reach 80°C.

Sterilisation Cycle

Stage 1 20°–121°C

Stage 2 <100°–121°C

Stage 3 121°–121°C

Stage 4 121°–80°C

Stage 1 The chamber heat-up time depends on the efficiency of the autoclave (air discharge/steam input) and the size of the load in the chamber. The time required for this stage is measured with a recording probe located in the air-discharge valve located in the base of the chamber.

Stage 2 The heat penetration time depends mainly on the volume of the individual containers, although the shape and the heat-transfer properties of the containers may affect this stage. The time required for the medium volume to reach 121°C is measured with thermocouples placed in the centre of the innermost container.

Volume (ml) in glass bottles	Time (mins)
100	19
500	18
1000	22
2000	27
5000	37

These times assume that agar media have been dissolved before autoclaving. It is also assumed that maximum exposure to steam is possible. Thus although the single 100ml bottle required 12 minutes to reach 121°C when placed in a crate with other bottles it required 19 minutes and when placed in the centre of stacked crates it required 30 minutes.

Stage 3 The holding time at 121°C depends on (i) the number of organisms originally present in the medium (ii) the fractional number of an organism presumed present after heating e.g. N = 0.001 equivalent to one bottle in every 1000 bottles heated becoming contaminated (iii) the thermal death rate constant of the presumed organism present at 121°C.

Stage 4 The cool-down time depends on the size of the load in the chamber and the heat loss rate from the autoclave. Water-sprays are used to accelerate cooling in commercial sterilisers but very careful control is required to avoid bottle fracture and the ingress of the cooling spray into the sterilised medium. The latter problem occurs when the vacuum formed in the head-space during cooling sucks contaminated cooling fluid up the thread of the cap and into the bottle.

Culture media autoclaves should be unlagged and of moderate chamber capacity only. Thermal locks on the doors should prevent them opening when the chamber temperature is above 80°C but even in these circumstances care should be taken to avoid sudden thermal shock when removing glass bottles of hot liquid from the autoclave. When screw-capped containers are placed in an autoclave the caps should be a half-turn free to allow the escape of heated air. When removed from the autoclave the caps are screwed down tightly after the contents have cooled to ambient temperature.

Sterilisation Checks

All autoclaves should be calibrated and checked at fixed periods of time to ensure that they are functioning efficiently. Physical measurements should be made on temperature and pressure readings, the quality of the steam should be checked, the efficiency of the 'near-to-steam' air traps in the base of the autoclave should be determined and the safety valves checked. Mandatory inspections of autoclaves as pressure vessels are normally carried out annually by specialists under instructions from insurers of such apparatus.

Biological indicators of sterilisation will demonstrate the ability of the autoclave to destroy bacterial spores. Such tests may be compulsory in certain countries.

Chemical indicators will show the temperature reached or exceeded and some will indicate the time held at the specified temperature. Under-autoclaving is usually self-evident because failure to destroy all the bacterial spores naturally present in dehydrated media (the 'bioburden') will allow growth to take place in the stored or incubated medium. Failure of sterilisation should always be suspected when contamination of prepared media occurs with sporing organisms.

Overheating Effects

Overheating is a common cause of pH drift, darkening, precipitation, poor gel strength and reduced bacteriological performance. These effects can also be produced if a concentrated 'pool' of ingredients at the bottom of the container is heated. All culture media should be in solution before sterilisation. This will reduce the occurrence of Maillard-type reactions (non-enzymatic browning) taking place in the medium.

Overheating effects will occur if agar media are allowed to gel in bottles and later steamed to melt the agar. They will also occur if molten media are held at 50°C for more than 3 hours before use. Agar media, with pH values at or below 5.0, are very sensitive to overheating in any form because the agar is hydrolysed and the gel strength fails.

Most of the difficulties in culture media sterilisation occur when large unit volumes of media (>2 litres) must be processed. The best solution to this problem is the use of a culture medium preparator. These semi-automatic processors, made by New Brunswick and other manufacturers overcome the problem of poor heat penetration of agar by a continuous stirring or agitation of the medium during the heating phase. Such preparators will significantly reduce the time required for sterilisation at 121°C or in some models at 134°C. They are strongly recommended because of their high efficiency and minimal damage to culture media.

Table of Faults and Possible Causes in Media Sterilisation

Fault
Wrong pH value.

Possible Causes
pH test carried out above 25°C.

Overheating through prolonged sterilisation, remelting or overlong period at 50°C.

Incomplete solution of medium.

Poor quality water or containers.

Dehydrated medium stored incorrectly or beyond the stated shelf-life.

Fault
Turbidity, precipitation.

Possible Causes
Poor quality water or containers.

Overheating or prolonged storage at 50°C.

pH value incorrect.

Incomplete solution.

Fault
Darkening.

Possible Causes
Overheating, incomplete solution or pH drift.

Fault
Soft gel.

Possible Causes
Agar not in solution, poor mixing, prolonged storage at 50°C.

Overheating at low pH values.

Error in weighing or overdilution with inoculum or media supplements.

Fault
Poor bacterial growth.

Possible Causes
Prolonged and excessive heating, incomplete solution.

Inhibitory substances in water or containers.

Darkening and pH drift.

PREPARATION OF STERILISED MEDIA

Liquid media which are sterilised in their final containers should be cooled down to room temperature as rapidly as possible. Screw caps should then be tightened.

Containers of agar media which have been sterilised should be placed in a 50°C water bath and the medium dispensed as soon as it reaches this temperature, or within a maximum of 3 hours in the bath. The medium should be mixed thoroughly, without bubble formation and aseptically dispensed into sterile containers. Do not expose dishes of agar media to sunlight; it causes excessive condensation on the lids and may cause the formation of inhibitory substances by photo-oxidation.

Heat-labile supplements should be added to the medium after it has cooled to 50°C. Allow the sterile supplement to come to room temperature before adding it to the agar medium. Cold liquids may cause agar to gel or form transparent flakes which can easily be seen in blood-enriched agar. Mix all supplements into the medium gently and thoroughly, then distribute into the final containers as quickly as possible.

Blood used for the preparation of blood agar should be as fresh as possible and should have been stored at 2–8°C (blood must not be frozen). Warm the blood in an incubator to about 35–37°C before addition to sterile molten agar base, which has been cooled to 40–45°C. Adequate mixing in a large head-space vessel is essential to ensure aeration of the blood. Poorly oxygenated blood plates are purplish in colour whereas properly aerated blood agar is cherry-red. Defibrinated blood is recommended for use rather than blood containing an anticoagulant.

STORAGE OF PREPARED MEDIA

The recommended shelf-life of prepared culture media varies considerably. Screw-capped bottles of nutrient broth and agar can be stored for 6 months at low ambient temperatures (12–16°C). It is important to store all media away from light.

Agar plates should be stored at 2–8°C in sealed containers to avoid loss of moisture.

DO NOT FREEZE.

Fresh media are better than stored media therefore avoid long storage times. Some very labile beta-lactam selective agents have very short active lives and media containing such substances should be used within a few days of preparation.

It is good laboratory practice to establish shelf-lives for all prepared media and date-stamp the containers or holders accordingly.

Loss of moisture from agar plates is a common cause of poor bacteriological performance. Do not pre-incubate all plates overnight as a sterility check. Only obviously wet plates require pre-inoculation drying. Ensure that all plates are incubated in a humid environment.

Examine prepared media before inoculation. Look for evidence of contamination, uneven filling or bubbles on surface of agar, colour changes, haemolysis and signs of dehydration such as shrinking, cracking and loss of volume. Discard any defective plates or tubes.

PRECAUTIONS IN THE USE AND DISPOSAL OF PREPARED MEDIA

It should be recognised that inoculation of culture media with bacteria, deliberately or accidentally, leads to very great numbers of organisms being produced. High concentrations of any organisms are potentially hazardous and must be disposed of safely by approved methods.

All infected specimens and inoculated culture media should be handled only by qualified personnel who have been trained in microbiological procedures. Such

staff should ensure that all specimens and cultures under their care are properly handled and finally autoclaved before disposal. Any apparatus used and contaminated must be safely disinfected or sterilised; this is particularly important when such apparatus must be serviced or passed out of the laboratory.

The environment in which microbiological cultures are handled must also be taken into account. Most countries have categories of organisms which are divided into those which may be handled in the general microbiological laboratory, those which require special laboratory conditions and for the most dangerous organisms a totally contained and highly protected environment. It may be a criminal offence not to observe these rules and regulations. When using culture media always label or identify the container with the specimen details before inoculation.

Inoculate the medium using aseptic techniques and incubate under the appropriate conditions.

Examine the medium after incubation for evidence of microbial growth and carry out the appropriate isolation and identification procedures.

PRECAUTIONS – DEHYDRATED MEDIA

Most of the products supplied by OXOID Limited have no known risks except those usually associated with fine powders. However, to prevent the risk of inhaling fine dust it is recommended that approved masks should be worn whilst handling dehydrated media.

Material Safety Data Sheets are available for individual products.

Dehydrated culture media supplied as powders, granules or tablets should not be eaten. Powders should not be inhaled because irritation of the upper respiratory tract may occur. To avoid mild skin rashes prevent prolonged contact with the powder and ensure excessive dust is not produced.

Powdered products, if spilt, can be collected and disposed of in the normal way. Any residue should be washed away with ample cold water.

HAZARDOUS PRODUCTS

There are a number of substances which contain toxic substances. These must be used in accordance with the product specific Material Safety Data Sheet. All relevant Risk and Safety Phrases are on the product label.

Media containing Sodium Azide
These products contain less than 1% sodium azide and have low toxicity. Some persons, however, have enhanced sensitivity to azide and therefore could react to accidental exposure to the product. Precautions must be taken to prevent ingestion or inhalation of the dust. Sodium azide reacts with many metals, especially copper, to produce explosive metal azides.

If Local or National legislation permits and disposal to sink is employed, use sufficient water to prevent the powder remaining in contact with pipework and drains. The same precaution applies to any biological solution which contains sodium azide as a preservative.

Products containing Barbitone
These products are labelled **POISON/TOXIC.**

They are subject to the Misuse of Drugs Act 1973. The supply is controlled and in the United Kingdom such products are available to bona fide laboratories only (orders must be signed by the head of department) or to establishments authorised to possess such products. Certain establishments are exempt under the regulations e.g. NHS hospital laboratories and other government departments but they will be asked to confirm this status.

Exports to some countries may require an import licence for the country of destination.

Cycloheximide
This compound, prepared in Supplement vials, reaches a concentration which is considered to be toxic and is labelled accordingly. However, when diluted out into the culture medium its concentration falls below the minimum level considered to be hazardous. It is important when reconstituting vials containing toxic levels of cycloheximide to ensure that the vial solution does not touch the skin and to prevent the creation of aerosols which would allow the compound to be inhaled. Wear personal protective equipment in accordance with the information in the Material Safety Data Sheet. The following First Aid procedures should be taken in cases of accident with any of the products described above:

FIRST AID PROCEDURES

Skin Contact
Remove all contaminated clothing; wash the affected areas thoroughly with soap and water. If any symptoms occur obtain medical advice.

Eye Contact
Irrigate thoroughly with water. Obtain medical advice.

Ingestion
Wash out mouth thoroughly with water. Give one pint of water to drink immediately. Obtain medical advice.

Inhalation
Remove person from area of exposure. Rest and keep warm. Obtain medical advice.

Spillage
Large quantities Wear protective overalls, gloves, eye protection and face mask. Collect the material into a suitable container and seal. Dispose according to local regulations. Wash away the residue with plenty of water.

Small quantities Wash away with large volumes of running water, using protective gloves.

USER-LABORATORY QUALITY CONTROL TESTS ON PREPARED MEDIA

Quality control tests should be carried out by the end-user laboratory to ensure that the performance characteristics of the medium are within specification and that the methodology of medium preparation is satisfactory.

Each lot/batch of prepared medium should be subjected to a minimal testing programme which will ensure that it is acceptable and will demonstrate a typical bacterial performance.

1 **pH value:** check that the pH of the prepared medium, when tested in final form at ambient temperature (25°C) lies within the range given on the product label. The medium should be discarded if the pH value lies outside the specified range.

2 **Sterility:** a representative sample of each lot/batch of medium should be incubated for 2–5 days at 35–37°C. As a general rule, for a lot of 100 or less units a 3–5% sample should be tested. For a larger lot, 10 random plates or tubes are taken. There should be no evidence of microbial growth after incubation. Discard all sterility samples when the tests have been completed.

3 **Growth performance:** test the growth support properties of the product by inoculating the medium with appropriate stock cultures and/or fresh isolates. Use a standard inoculation procedure and examine the quantitative and qualitative results obtained. If testing new lots/batches of media, inoculate old and new lots in one test and compare the performance of the two lots side by side.

4 **Stability:** periodically perform the above procedures on stored prepared media in order to determine whether the storage conditions will give optimal results.

NOTE: If a medium does not perform to expectations and all the manufacturer's recommendations have been followed, then the following steps should be taken:

1 Record the nature of the problem and the method of preparation of the medium.

2 Note the lot/batch number and the date it was received.

3 Call the Technical Support department of the company.

SPECIAL FIELDS OF CULTURE MEDIA APPLICATION

EXAMINATION OF CLINICAL AND VETERINARY SAMPLES

In both clinical and veterinary microbiology the purpose of examining samples of tissue, fluids or excreta is to isolate and identify pathogenic organisms.

Although both fields of investigation have common interests and common organisms, they are separate specialist activities. Reference should be made to the appropriate specialist publications in either field to obtain specific guidance.

It should be stressed that every specimen must be evaluated, many laboratories cannot cover the whole microbiological field, the various infective agents should be taken into consideration and, if necessary, material referred to the appropriate reference laboratory.

Poor specimen samples can only yield poor or misleading results. It is important that personnel collecting or taking samples are instructed by the laboratory to prevent faulty collection procedures.

Satisfactory samples, collected without extraneous contamination and before antimicrobial therapy should be transferred to the laboratory with minimal delay. If transportation is required then appropriate transport media should be used to protect delicate organisms. Where quantitative results are important e.g. urine cytology and bacteriology, or where commensal overgrowth should be prevented, refrigeration of samples at 2–8°C is essential.

All samples should be clearly labelled and sent in leak-proof, satisfactory containers. Sealed, transparent plastic bags, containing the sample container and the request form attached to but not inside the plastic bag, is the most acceptable method of sending pathological samples to the laboratory.

BLOOD CULTURES

A full description of the Oxoid Signal Blood Culture System and the Isolator Blood Culture System is given on pages 8–1 to 8–10.

Examination of blood for infectious agents is one of the most important and often most urgent examinations requested. All the various systems of blood culturing require blood samples to be collected with scrupulous care to avoid extravenous contamination. The blood/broth medium should be subcultured to appropriate media either at fixed time intervals or whenever changes in appearance of the medium are noted e.g. turbidity, darkening, lysis etc. Subculture after 24 hours incubation, regardless of appearance, is recommended to detect early evidence of bacteraemia. All subcultures must be made with great care to avoid contaminating the blood/broth medium.

Associated pathogens
Staphylococci (coagulase positive and negative)
Streptococci (alpha/beta/non-haemolytic strains)
Coliform organisms (including other enteric organisms)
Non-fermentative organisms (*Pseudomonas* and *Acinetobacter* species)
Anaerobes (*Clostridia, Bacteroides, Fusiformis* species and anaerobic cocci)
Neisseria species
Haemophilus influenzae
Brucella species
Immune-compromised patients are subject to blood-borne infections by any opportunistic organism: mycobacteria, fungi and rare/exotic organisms should be anticipated.

Commensal organisms
None.

CEREBROSPINAL FLUID (CSF)

It is very important that all samples of CSF are examined with minimal delay. A description of the appearance of the sample must be made e.g. colour, clarity, clots etc., the cells, protein and sugar content should then be measured. The following results are indications of infection:

raised polymorphs/low sugar – indicates bacterial infection

raised lymphocytes/normal sugar – indicates viral infection

raised lymphocytes/high protein/low sugar – indicates mycobacterial infection.

If a fibrin clot is present then particular attention should be paid to *Mycobacterium tuberculosis.*

Cell counts are of little validity when clots are present.

Centrifuge a portion of the CSF and make three films of sufficiently small area so that the whole may be examined under the microscope. Stain one film by Gram's stain, one by Leishmann or Giemsa stain and one by an acid-fast bacilli stain. In purulent samples *Haemophilus influenzae* may be difficult to see under Gram's stain. Carbol-thionin or a similar nucleic-acid stain may be helpful to see the bacteria in such circumstances.

Inoculate a portion of the centrifuged sample (taking suitable aseptic precautions) on blood agar (incubate aerobically and anaerobically), 'chocolate' Columbia Agar (incubate in a 5% CO_2 atmosphere) examine after 18–24 hours incubation at 35°C. Carry out antimicrobial susceptibility tests on any organisms isolated. Select appropriate antimicrobials for blood/brain infections. Culture for *Mycobacterium tuberculosis* if the examination results indicate tuberculosis.

Direct tests to identify common bacterial antigens in CSF are available.

Associated pathogens
Haemophilus influenzae
Neisseria meningitidis
Streptococcus pneumoniae
Mycobacterium tuberculosis
Listeria monocytogenes

Nocardia species and *Bacillus Cryptococcus neoformans* Coliform bacilli, *Pseudomonas* species and Group B streptococci occur in neonates.

Patients involved in surgical manipulations e.g. shunts, valves etc., can become infected with *Staphylococcus epidermidis* and micrococci.

Commensal organisms
None.

SPUTUM
Samples of sputum are often the poorest samples received. The ideal of obtaining discharge from the bronchial tree, without contamination from saliva, is seldom achieved.

Obvious samples of saliva should be rejected. Washing the sample with sterile saline to separate purulent material may be necessary to reduce salival contamination. Homogenisation with Sputosol (SR89) will also help assess the significant flora which may be localised in one small part of the sample. Make films for a Gram's stain and an acid-fast bacilli stain. Inoculate blood agar media and incubate in 5% CO_2 atmosphere at 35°C overnight.

A MacConkey Agar plate will help distinguish the coliforms and streptococci frequently found in sputum. If legionellosis is suspected inoculate Legionella Medium (CM655 + SR110 + SR111).

If mycetoma or other fungal diseases are suspected inoculate Sabouraud Dextrose Agar (CM41) or Dermasel Agar Base (CM539+ SR75).

Associated pathogens
Staphylococcus aureus
Streptococcus pneumoniae
Haemophilus influenzae
Coliform bacilli
Klebsiella pneumoniae
Pasteurella species/*Yersinia* species
Mycobacterium tuberculosis
Branhamella catarrhalis
Mycoplasma species
Legionella species
Candida/Aspergillus/Histoplasma/Cryptococcus/ Blastomyces species

Commensal organisms
Staphylococcus epidermidis, micrococci, non-pathogenic neisseria, *Streptococcus viridans*, small numbers of *Candida* and coliform bacilli.

EAR, NOSE AND THROAT SWABS
The ENT department will send good samples to the laboratory but samples taken elsewhere may be less satisfactory and care should be taken that staff are instructed on how to take satisfactory ENT swabs.

Ear swabs: make films and stain with Gram's solutions and with methylene blue if diphtheria is suspected. Inoculate blood agar plates and incubate aerobically and anaerobically for 18–24 hours at 35°C. Inoculate tellurite medium if the swab is from a child of school age or if diphtheria is suspected for other reasons.

Associated pathogens
Staphylococcus aureus
Streptococcus pyogenes
Haemophilus species
Corynebacterium diphtheriae
Pseudomonas aeruginosa
Coliform bacilli
Bacteroides/Fusiformis species
Fungi

Commensal organisms
Micrococci, diphtheroids and *Staphylococcus epidermidis.*

Nose swabs: anterior nasal swabs or pernasal swabs may be sent depending on the organisms suspected. Direct films are of little value. Inoculate blood agar and tellurite media, incubate 18–24 hours at 35°C. Pernasal swabs for *Bordetella pertussis* should be inoculated on to Charcoal Agar (CM119 + SR82).

Associated pathogens
Staphylococcus aureus
Streptococcus pyogenes
Neisseria meningitidis
Bordetella pertussis
Haemophilus species
Corynebacterium diphtheriae

Commensal organisms
Diphtheroids, *Stapylococcus epidermidis,* non-pathogenic neisseria, *Bacillus* species, small numbers of coliform bacilli.

Throat swabs: make a film and stain with dilute carbol-fuchsin, examine for Vincent's organisms, yeasts and mycelium.

Inoculate blood agar and incubate aerobically and anaerobically for 18–24 hours at 35°C. Inoculate tellurite medium and incubate for 48 hours at 35°C.

Associated pathogens
Streptococcus pyogenes
Corynebacterium diphtheriae
Corynebacterium ulcerans
Staphylococcus aureus
Neisseria meningitidis
Candida albicans
Borrelia vincenti

Commensal organisms
Streptococcus viridans, Staphylococcus epidermidis, diphtheroids, *Streptococcus pneumoniae, Haemophilus influenzae* non-type B, non-pathogenic neisseria.

URINE
Specimens of urine for microbiological examination are usually 'mid-stream' samples, more rarely catheter collected samples or supra-pubic aspirations. All samples should be delivered quickly to the laboratory, or preserved for short periods at 2–8°C, or a small amount of boric acid can be added.

Dip Slides have the advantage that they can be immersed in fresh urine or the patient can micturate directly on to the agar surface of the Dip Slide. Thus the bacterial colonies seen after transport and incubation reflect accurately the original microbial ecology.

Examination of urine includes counting white cells, red cells and urinary casts, estimating the number of bacteria per ml and identifying the organisms grown.

Samples of urine can be inoculated on to MacConkey Agar and CLED Agar using a calibrated loop (0.01ml) or filter paper inoculation. Incubate overnight at 35°C, and count the number of colonies developed.

<20 colonies = $<10^4$ orgs/ml
20–200 colonies = 10^4-10^5 orgs/ml
>200 colonies = $>10^5$ orgs/ml

The question of significance of growth depends on the flora grown and the clinical history of the patient. The criteria proposed by Kass (significance = $>10^5$ cfu/ml) for asymptomatic patients does not apply universally to all patients.

Associated pathogens
Escherichia coli
Enterobacter and *Proteus* species
Staphylococci (coagulase positive and negative)
Enterococcus
Mycobacterium tuberculosis

Commensal organisms
When in doubt contact the physician or repeat the sample.

PUS AND WOUND SWABS

Samples of pus or properly taken swabs of wound exudates should be sent quickly to the laboratory.

Pus samples should be diluted with sterile saline to detect the 'sulphur granules' of *Actinomyces israelii.* Inoculate the granules on to blood agar and incubate aerobically and anaerobically. To avoid *Proteus* species spreading across the plates use chloral hydrate in one of the plates or take equivalent precautions. Wilkins-Chalgren Anaerobic Agar (CM6l9 + SR107 or SR108) can be used to isolate anaerobes. Inoculation into thioglycollate broth is helpful to enrich the growth of anaerobes and aerobes.

Examine Gram-stained films and acid-fast bacilli stained films. Superficial wounds may be infected with atypical mycobacteria (*Myco. marinum*, *Myco. ulcerans*, *Myco. chelonei*), culture on Lowenstein-Jensen medium and incubate at 30°C. Wounds from burns, although infected with *Staphylococcus aureus* and *Streptococcus pyogenes*, may also be heavily colonised with Gram-negative organisms – especially *Pseudomonas* species. Examination of films and inoculation on to blood agar plates containing Staph/Strep Supplement SR70, chloral hydrate or phenethyl alcohol should help separate the infecting organism. Incubate aerobically and anaerobically at 35°C.

Examine the plates soon after removal from the incubator because *Proteus* species become more motile at room temperature.

Associated pathogens
Staphylococcus aureus
Streptococcus pyogenes
Anaerobic cocci
Clostridia species
Bacteroides species
Pasteurella species
Yersinia species
Actinomyces species
Mycobacterium species
Bacillus anthracis
Listeria monocytogenes
Proteus and *Pseudomonas* species
Nocardia and other fungi

Commensal organisms
Pus – none
Wound swabs – small numbers of skin commensal organisms.

EYE SWABS (purulent discharges)
Eye discharge swabs should arrive in transport media but preferably the eye discharge should be sampled directly on to culture media.

Examine smears for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, using Gram's stain and Giemsa stain or immuno-fluorescent reagents.

Inoculate blood agar plates and incubate aerobically and anaerobically at 35°C overnight.

Inoculate a Columbia 'chocolate' blood agar and incubate in a 5% CO_2 atmosphere at 35°C overnight. Prolong the incubation for 48 hours if the Gram film is doubtful.

Associated pathogens
Staphylococcus aureus
Streptococcus pneumoniae
Neisseria gonorrhoeae
Haemophilus species
Chlamydia trachomatis
Moraxella species
Corynebacterium diphtheriae
Pseudomonas aeruginosa
Coliform organisms

Commensal organisms
Staphylococcus epidermidis
Micrococcus species
Diphtheroids

FAECES, FAECAL AND RECTAL SWABS
Rectal swabs are of the least value compared with samples of faeces or faecal swabs. All samples should be sent to the laboratory quickly or placed in transport media.

There is a very wide range of culture media available to cultivate the growing list of enteric pathogens. It would not be cost-effective to use them indiscriminately therefore the clinical history of the patient is essential to focus attention on the most likely organisms.

Salmonellae* and *Shigellae: inoculate one or more enrichment media (selenite/tetrathionate/RV broths) and at least two isolation media, one of which must be able to support the growth of shigella (DCLS, DCA, Hektoen, Modified SS, XLD). Incubate for 18–24 hours at 35°C although tetrathionate broth and RV broth can be incubated at 43°C to increase selectivity for salmonellae. Subculture on to appropriate media.

Enterotoxigenic *Escherichia coli* (ETEC): inoculate MacConkey Agar and MacConkey Sorbitol Agar CM813. Look for non-sorbitol fermenting colonies indicative of *Escherichia coli* O157:H7; confirm identity with serological tests. Look for *Staphylococcus aureus* also on MacConkey Agar in case the disease is staphylococcal enterocolitis.

Campylobacter: inoculate Campylobacter Selective media made with one of the various selective supplements available.

Vibrios: *V. cholerae* or *V. parahaemolyticus* may be suspected. Inoculate alkaline peptone water and TCBS Agar CM333.

Yersinia: *Y. enterocolitica* may be isolated on Yersinia Selective Agar Base (CM653 + SR109). Inoculate the medium and incubate for 18–24 hours at 32°C.

Clostridium perfringens: inoculate blood agar and incubate anaerobically (and aerobically as a control). Inoculate two tubes of Cooked Meat Broth and heat one at 80°C for 30 minutes to detect heat-resistant spores. Subculture to blood agar and incubate anaerobically and aerobically.

Aeromonas: *A. hydrophila* and *A. sobria* are associated with enteritis of children and adults. Inoculate Aeromonas Medium Base (Ryan) CM833 + SR136 or Blood Agar containing 20mgm per litre of Ampicillin. Incubate 18–24 hours at 35°C.

Clostridium difficile: when this organism is isolated from antimicrobial-associated-colitis it is considered to be a pathogen. It can be found fairly commonly in infant stools where it is usually non-toxigenic. Inoculate alcohol-treated faeces on Clostridium Difficile Agar Base (CM601 + SR96) and incubate anaerobically at 35°C for 18–24 hours.

Associated pathogens
Bacillus cereus
Plesiomonas shigelloides
Clostridium botulinum

Commensal organisms
Coliform bacilli, *Proteus* species, *Pseudomonas* species, *Bacteroides* species and many *Clostridium* species.

SEXUALLY TRANSMITTED DISEASE SWABS
STD samples may come from the eye, throat, rectum, cervix, vagina or urethra.

Eye swabs: look for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* as previously described.

Throat swabs: look specifically for *N. gonorrhoeae.*

Vaginal/cervical swabs: examine a Gram's stained smear for *N. gonorrhoeae* and a 'wet' slide preparation for *Trichomonas vaginalis* and for 'clue cells' diagnostic for *Gardnerella vaginalis*. Yeast cells may be seen in either preparation. To isolate *G. vaginalis* inoculate Columbia Blood Agar Base containing 10% human, rabbit or horse blood plus G. vaginalis Selective Supplement (SR119). Incubate at 35°C in a 7% CO_2 atmosphere for 48 hours.

Urethral swabs: as well as *N. gonorrhoeae*, include *C. trachomatis* in the smear examination using Giemsa stain or a specific immunofluorescent reagent.

Inoculate all swabs on Thayer-Martin Medium (CM367 + SR90 + SR91 or SR101) or on Modified New York City Medium (CM367 + SR105+ SR95 or SR104). Incubate in a 5% CO_2 atmosphere at 35°C for 24–48 hours. Sabouraud Dextrose Agar or Dermasel Agar can be inoculated if *Candida* are suspected.

PUERPERAL INFECTIONS
High vaginal swabs from such conditions should be examined carefully for *Clostridium perfringens*. Non-sporing, square-ended Gram-positive rods which appear to be capsulated may be seen in the Gram stained film and should reported to the physician immediately.

Inoculate blood agar plates and incubate aerobically and anaerobically at 35°C for 18–24 hours.

EXAMINATION OF FOOD AND DAIRY PRODUCTS

There is no general agreement on methods for the laboratory examination of foods and dairy products.

The standard reference books used are:
Compendium of Methods for the Microbiological Examination of Foods by the American Public Health Association. Washington D.C. 1976.

Bacteriological Analytical Manual 8th Edn. Revision A by the Association of Official Analytical Chemists. Washington D.C. 1978.

Microorganisms in Foods Vols. 1 & 2. by the International Commission on Microbiological Specifications for Foods. Toronto University Press. 1988 with Revision.

In Europe, the Codex Alimentarius Commission is considering standard methods, aided by published standards from the International Organization for Standardization (ISO).

The bacteriological examination of food and dairy products falls into one or more of the following four categories:

1 **Total Viable Count:** this is an attempt to measure the total number of bacteria, yeasts and moulds in a product by inoculating dilutions of suspensions of the sample into various culture media and incubating them for fixed periods at temperatures varying from 22°C to 55°C. The resulting colony counts are then calculated as organisms per gram of product. The results obtained are compared with expected figures and the product is passed or failed. It is not an accurate process and fairly gross changes in numbers are looked for which indicate unsatisfactory raw materials, processing or storage conditions.
2 **Indicator Organism Count:** specific organisms are sought, most often coliforms (lactose-fermenters) or Enterobacteriaceae (glucose-fermenters) using selective media. See section on Violet Red Bile Agar and Violet Red Bile Glucose Agar. These organisms indicate the standard of hygiene used in the manufacture of the food products.
3 **Detection of Specific Spoilage organisms:** spoilage organisms are usually associated with

taints and off-flavours in stored products. They are the major factor in determining the shelf-lives of food products and are now considered to be of more relevance than total viable counts. Moulds and psychrotrophic Gram-negative rods are specifically sought, using selective culture media and low temperature incubation.

4 **Detection of Food Poisoning Organisms:** Hazard analysis critical control point technique (HACCP) is a systematic approach to hazard identification, assessment and control. The hazards are determined, the critical control points of those hazards are identified and procedures to monitor the critical control points are established. An HACCP audit is an essential stage in the implementation of this process. [ICMSF (1989) ''Micro-organisms in Foods, 4. Application of hazard analysis critical control point (HACCP) system to ensure microbiological safety and quality''. Blackwell Scientific Publications, Oxford.]

MEDIA FOR FOOD AND DAIRY MICROBIOLOGY

Groups of Micro-organisms/Test	Purpose	Media	Code
Anaerobes	Cultivation and enumeration of anaerobic micro-organisms e.g. clostridia, lactobacilli and streptococci RCM is recommended as the diluent in the determination of viable counts of anaerobes	Cooked Meat Medium Liver Broth Perfringens Agar Base (OPSP) Perfringens Agar Base (TSC/SFP) Reinforced Clostridial Agar Reinforced Clostridial Medium (RCM)	CM81 CM77 CM543 CM587 CM151 CM149
	Detection and enumeration of thermophilic anaerobes causing sulphide spoilage	Iron Sulphite Agar	CM79
	Examination of canned food samples for anaerobic bacteria. Diagnostic tests for the identification of *Clostridium* species e.g. *Cl. perfringens*	Crossley Milk Medium	CM213
	Confirmation of *Cl. perfringens* by Nagler Test	Blood Agar Base Fildes Extract Egg Yolk Emulsion	CM55 SR46 SR47
	Enterotoxin detection	PET-RPLA	TD900
	Selective isolation of anaerobic organisms from dehydrated or frozen foods	Schaedler Anaerobe Agar Schaedler Anaerobe Broth Wilkins-Chalgren Anaerobe Agar Wilkins-Chalgren Anaerobe Broth	CM437 CM497 CM619 CM643
	Isolation of *Cl. difficile*	Clostridium difficile Agar Base Clostridium difficile Selective Supplement CDMN Supplement	CM601 SR96 SR173
Bacillus cereus	Detection of *B. cereus* by lecithinase activity after destroying non-spore-forming organisms from foods e.g. rice, pepper and milk	Egg Yolk Emulsion Nutrient Agar Bacillus cereus Selective Medium	SR47 CM3 CM617 +SR99
	Enterotoxin detection	BCET-RPLA	TD950
Campylobacter	For selective isolation of thermophilic *Campylobacter* species Detection of coliform organisms	**Campylobacter Selective Media** **Bases:** Blood Agar Base No.2 *or* Blood Free Campylobacter Selective Agar Base *or* Campylobacter Agar Base *or* Campylobacter Agar Base (Karmali) *or* Columbia Agar **Supplements:** Campylobacter Growth Supplement 1 vial per 500ml of medium Campylobacter Selective Supplement (Blaser/Wang) 1 vial per 500ml of medium Campylobacter Selective Supplement (Butzler) 1 vial per 500ml of medium Campylobacter Selective Supplement (Skirrow) 1 vial per 500ml of medium	 CM271 CM739 CM689 CM935 CM331 SR84 SR98 SR85 SR69

Groups of Micro-organisms/Test	Purpose	Media	Code
Campylobacter *cont.*		Campylobacter Selective Supplement (Preston) 1 vial per 500ml of medium	SR117
		CCDA Selective Supplement (for blood-free medium) 1 vial per 500ml of medium	SR155
		CAT Selective Supplement	SR174
		Campylobacter Selective Supplement (Karmali) 1 vial per 500ml of medium	SR167
		Laked Horse Blood – use at 7%	SR48
Coli-aerogenes group of the Entero-bacteriaceae	Presumptive coliform tests	Lactose Broth	CM137
		Lauryl Tryptose Broth (Lauryl Sulphate Broth)	CM451
		MacConkey Broth (Purple)	CM5a
		Violet Red Bile Lactose Agar	CM107
		Chromgenic *E. coli*/coliform Medium	CM956
	Confirmation of the presence of *Esch. coli* at 44°C.	Brilliant Green Bile (2%) Broth	CM31
		TBX Agar	CM945
		Tryptone Bile Agar	CM595
	The Eijkman test in MacConkey Broth may also be used.	MacConkey Broth	CM5
	Differentiation between lactose and non-lactose fermenting organisms	China Blue Lactose Agar	CM209
		Endo Agar Base	CM479
	Differentiation and enumeration of coliform bacilli	Desoxycholate Agar	CM163
		Eosin Methylene Blue Agar (Levine)	CM69
		Violet Red Bile Lactose Agar	CM107
		Violet Red Bile Glucose Agar	CM485
		Sorbitol MacConkey Agar	CM813
	Detection of *E. coli*	MUG Supplement	BR71
	Detection of *E. coli* O157:H7	*Escherichia coli* 0157 Latex Test A latex agglutination test for the identification of *Esch. coli* serogroup O157	DR620
	Confirmation of presumptive coliform tests	Endo Agar Base	CM479
		Basic Fuchsin Indicator	BR50
		Eosin Methylene Blue Agar (Levine)	CM69
	Differentiation of the coli-aerogenes group: (a) Methyl Red and Voges-Proskauer tests. (b) Citrate utilisation (c) Production of Indole	MRVP Medium	CM43
		Simmons Citrate Agar	CM155
		Lauryl Tryptose Broth (Lauryl Sulphate Broth)	CM451
		Tryptone Water	CM87
Enterobacteriaceae (see also coli-Aerogenes group and Salmonella and Shigella)	Cultivation of Enterobacteriaceae: Resuscitation of sub-lethally impaired cells e.g. in preserved foods, prior to enrichment	Buffered Peptone Water	CM509
		Tryptone Soya Broth	CM129
	Enrichment medium for Enterobacteriaceae in the examination of foods and animal feed stuffs	EE Broth (Buffered Glucose – Brilliant Green Bile Broth)	CM317
	Selective enumeration of Enterobacteriaceae	MacConkey Agar No.3	CM115
		Tergitol-7 Agar	CM793
		Violet Red Bile Glucose Agar	CM485
	Differentiation of the Enterobaceriaceae	SIM Medium	CM435
		Triple Sugar Iron Agar	CM277
		Kligler Iron Agar	CM33
	Detection of urease-producers e.g. *Proteus* group	Urea Agar Base (Christensen Agar Base)	CM53

Groups of Micro-organisms/Test	Purpose	Media	Code
Enterococci	Selective enumeration of Lancefield group D streptococci	Azide Blood Agar Base	CM259
		Azide Dextrose Broth	CM868
		Ethyl Violet Azide Broth	CM869
		Kanamycin Aesculin Azide Agar	CM481
		K-F Streptococcus Agar	CM701
		MacConkey Agar No.2	CM109
		Slanetz and Bartley Medium (Enterococcus Agar)	CM377
	Latex agglutination test	Streptococcal Grouping Kit	DR585
Gelatin-liquefying organisms	Gelatin liquefaction is used as an aid in the identification of certain micro-organisms e.g. *E. faecalis* var. *liquefaciens*	Staphylococcus Medium 110	CM145
		Nutrient Gelatin (CM135a)	CM635
Lactobacilli	Selective isolation and enumeration of lactobacilli e.g. from meats, yoghurt etc.	MRS Agar/Broth	CM361/CM359
		Tomato Juice Agar	CM113
		Rogosa Agar	CM627
		Orange Serum Agar	CM657
Lecithinase-producing organisms	Lecithin activity is used in the identification of certain organisms, e.g. some *Bacillus* species	Egg Yolk Emulsion	SR47
		Nutrient Agar	CM3
Lipolytic organisms	Isolation of contaminating lipolytic organisms from milk, butter, cream, etc. Also used for the examination of activity of moulds in mould ripened cheese	Tributyrin Agar	PM4
Listeria monocytogenes	Selective isolation	Buffered Listeria Enrichment Broth	CM897
		Listeria Selective Enrichment Supplement	SR141
		Fraser Broth	CM895
		Fraser Supplement	SR156
		Half-Fraser Supplement	SR166
		Listeria Selective Agar Base (Oxford)	CM856
		Listeria Selective Supplement	SR140
		Listeria Enrichment Broth	CM862
		Listeria Selective Enrichment Supplement	SR141
		Listeria Selective Enrichment Supplement (modified) with 10mg/l Acriflavine	SR149
		Listeria Enrichment Broth Base (UVM formulation)	CM863
		Listeria Primary Selective Enrichment Supplement (UVM)	SR142
		Listeria Secondary Selective Enrichment Supplement (UVM II)	SR143
		Listeria Rapid Test	FT401
		PALCAM Agar Base	CM877
		PALCAM Selective Supplement	SR150
Micrococci	Enumeration and differentiation of lactose and non-lactose fermenting organisms including micrococci	China Blue Lactose Agar	CM209

Groups of Micro-organisms/Test	Purpose	Media	Code
Plate Count	Colony count for indicating the efficiency of water treatment processes, or suitability of water for food preparation. Nutrient gelatin is used for the plate count of psychrophilic organisms e.g. *Pseudomonas* species and for testing gelatinase activity	Nutrient Gelatin (CM135a)	CM635
		Tryptone Glucose Extract Agar	CM127
		Tryptone Soya Agar	CM131
		Yeast Extract Agar	CM19
		Plate Count Agar	CM325
		Standard Plate Count Agar (APHA)	CM463
		Milk Plate Count Agar with antibiotic free skim milk	CM681
		Milk Agar	CM21
		PPCT Selective Supplement	SR159
Salmonellae and Shigellae	Pre-enrichment procedures prior to isolation	Buffered Peptone Water	CM509
		Mannitol Selenite Broth Base	CM399
	Enrichment procedures prior to isolation	Muller-Kauffmann Tetrathionate Broth Base	CM343
		Selenite Broth Base	CM395
		Sodium Biselenite	L121
		Tetrathionate Broth Base	CM29
	Isolation and identification of *Salmonella* and *Shigella* species	Rappaport-Vassiliadis (RV) – Enrichment Broth	CM669
		Rappaport Vassiliadis Soya (RVS) Peptone Broth	CM866
		Selenite Cystine Broth Base	CM699
		Tetrathionate Broth USA	CM671
		Bismuth Sulphate Agar	CM201
		Brilliant Green Agar	CM263
		Brilliant Green Agar (Modified) (Edel-Kampelmacher Medium)	CM329
		Sulphamandelate Supplement	SR87
		DCLS Agar	CM393
		Desoxycholate Citrate Agar (Hynes)	CM227
		Hektoen Enteric Agar	CM419
		XLD Medium	CM469
		MLCB Agar	CM783
	Identification of salmonella and shigella.	Kligler Iron Agar	CM33
		Lysine Iron Agar	CM381
		Triple Sugar Iron Agar	CM277
	Salmonella Rapid Test	Elective Medium	CM857
		Rapid Test	FT201
		Latex Test	FT203
Staphylococci	Isolation and differentiation of pathogenic staphylococci	Baird-Parker Agar Base	CM275
		Egg Yolk Tellurite Emulsion	SR54
		Egg Yolk Emulsion	SR47
		Giolitti-Cantoni Broth	CM523
		Staphylococcus Medium No.110	CM145
		Staph/Strep. Supplement	SR70
		Vogel-Johnson Agar	CM641
		Mannitol Salt Agar (Chapman Medium)	CM85
	Selective enumeration of the total staphylococci count from foodstuffs	Baird-Parker Agar Base	CM275
		Egg Yolk Emulsion	SR47
		RPF Supplement	SR122
	Differentiation of staphylococci:		
	(a) DNase production	DNase Agar	CM321
	(b) Phosphatase production	Blood Agar Base	CM55
	(c) Coagulase production	Staphylase Test	
		A test kit for the identification of *Staphylococcus aureus*:	DR595
		Staphytect	DR650

Groups of Micro-organisms/Test	Purpose	Media	Code
Streptococci and Enterococci	Selective isolation of streptococci from dairy products containing mixed flora. Tryptose Phosphate Broth is used with added azide and agar (APHA)	M17 Agar	CM785
		Edwards Medium (modified)	CM27
		Tryptose Phosphate Broth	CM283
		Kanamycin Aesculin Azide Agar Base	CM591
		Kanamycin Sulphate Supplement	SR92
		K-F Streptococcus Agar	CM701
	Isolation of streptococci and staphylococci	Columbia Blood Agar Base	CM331
		Streptococcus Supplement (Colistin-Oxolinic Acid)	SR126
		Staph/Strep Supplement	SR70
	Latex agglutination	Streptococcal Grouping Kit	DR585
Thermophilic 'flat-sour' micro-organisms	Detection and enumeration of 'flat-sour' organisms in canned foods, sugar, etc.	Dextrose Tryptone Agar	CM75
		Dextrose Tryptone Broth	CM73
		Shapton Medium	CM270
	Detection and emumeration of flat-sour organisms (*Bacillus stearothermophilus)* in canned milk products and sugar	Tryptone Glucose Extract Agar	CM127
Thermophilic Anaerobes	Detection and enumeration of thermophilic anaerobes causing sulphide spoilage e.g. *Clostridium sporogenes*	Iron Sulphite Agar	CM79
Viable Organisms	General viable count and cultivation of micro-organisms	Blood Agar Base	CM55
		Nutrient Agar	CM3
		Nutrient Broth No.2	CM67
		Plate Count Agar	CM325
		Standard Plate Count Agar (APHA)	CM463
		Tryptone Soya Agar	CM131
		Tryptone Soya Broth	CM129
		Yeast Extract Agar	CM19
		Milk Agar	CM21
		Post-Pasteurisation Contamination Test (PPCT)	SR159
	General cultivation	Nutrient Broth No.2	CM67
		Maximum Recovery Diluent (Peptone Salt Broth)	CM733
Vibrios	Isolation and identification of the Vibrionaceae	Cholera Medium TCBS	CM333
		Aeromonas Medium Base (Ryan)	CM833
		Ampicillin Selective Supplement	SR136
	For presumptive identification of *Vibrio* species	0129 Discs – 10µg per disc	DD14
		0129 Discs – 150µg per disc	DD15
Yeasts and Moulds	Cultivation and enumeration of yeasts and moulds	Malt Extract Agar	CM59
		OGYE Agar	CM545
		Potato Dextrose Agar	CM139
		Rose-Bengal Chloramphenicol Agar	CM549
		Yeast and Mould Agar	CM920
	Enumeration of moulds and yeasts	Dichloran-Glycerol (DG18) Agar Base	CM729
		Chloramphenicol Selective Supplement	SR78
		DRBC Agar Base	CM727
		Chloramphenicol Selective Supplement	SR78

Groups of Micro-organisms/Test	Purpose	Media	Code
Plant Hygiene and Food Sample Dilution	Diluent or rinse in bacteriological examination of food products, plant and apparatus	Ringer Solution Tablets	BR52
	Solvent diluent solution for calcium alginate swabs	'Calgon' Ringer Tablets	BR49
	Chlorine-neutralising Ringer Solution to counteract the bactericidal effect of hypochlorites or other chlorine sources	Thiosulphate Ringer Tablets	BR48

STERILITY AND PHARMACEUTICAL PRODUCTS

The safety tests of pharmaceutical and biological products include procedures to measure:

1 the absence of viable micro-organisms (sterile products)

2 the absence or presence within limits of specific organisms (clostridia, salmonella, pseudomonads, coliform bacilli, staphylococci and streptococci)

3 the microbial flora of raw materials and natural substances (the 'bioburden').

Before carrying out these tests it is important that the appropriate reference texts are consulted for the full descriptions of the methods required. There are no universally approved standards and each country has national standards which must be followed.

Examples of publications which offer complete, detailed test procedures and interpretations of the results obtained are:

The United States Pharmacopoeia 23 and The National Formulary 18. Rockville Md. 1995.

U.S. Code of Federal Regulations, Food and Drugs Parts 170–499. 1997.

Official Methods of Analysis of the AOAC 16th Edn. Washington D.C. 1998 vol. I and II.

British Pharmacopoeia 1993 Vol. I and II (HMSO). 1998.

European Pharmacopoeia 3rd Edn. Sainte-Ruffine, France 1997 + 1998 Supplement Part II.

WHO International Pharmacopoeia 3rd Edn. Geneva 1994.

The Pharmacopoeia of Japan, Tokyo. Society of Japanese Pharmacopoeia. 12Ed. JPXII 1991.

Many pharmaceutical and biological reagents contain preservatives and, when testing them for the presence of viable organisms, it is important to add neutralising agents to the recovery media to overcome residual antimicrobial effects. Some sterility test media contain antagonists to specific preservatives in their formulation.

Preservative	**Neutralising Agent**
Halogens	1% sodium thiosulphate
Aldehydes	2% sodium sulphite
Hexachlorophenes and Quaternaries	5% Tween 80 1% lecithin
Phenols/alcohols	Dilute 1:100 with nutrient broth.

To overcome the bacteriostatic effects of antimicrobial compounds, a filtration technique is used in which the product is passed aseptically through a 0.22 micron membrane filter. The filters are washed with sterile diluent to remove residues of antimicrobials on the filter; they are then cut with sterile scissors and distributed aseptically among various media. This technique can also be used for other preservative compounds.

Oily substances and some insoluble powders will require treatment with sterile Tween 80 to make them suitable for microbial examination.

Incubation of inoculated anaerobic and aerobic media should be extended up to 7 days at 35°C before final examination and subculture. Incubation at 30–32°C for the same period is usually recommended for yeasts and moulds.

MEDIA FOR PHARMACEUTICAL LABORATORY PROCEDURES

Test	Organism	Culture Media	Code
Sterility Testing		Blood Agar Base	CM55
		Brain Heart Infusion	CM375/CM225
		Clausen Medium	CM353
		*Cooked Meat Medium	CM81
		Dextrose Tryptone Agar	CM75
		Dextrose Tryptone Broth	CM73
		Liver Broth	CM77
		Malt Extract Agar	CM59
		Malt Extract Broth	CM57
		Membrane Media (MM Series)	
		*Nutrient Broth No. 2	CM67
		Reinforced Clostridial Medium	CM151/CM149
		*Sabouraud Liquid Medium	CM147
		*Tryptone Soya Broth	CM129
		*Thioglycollate Broth U.S.P. Alternative	CM391
		*Thioglycollate Medium (Brewer)	CM23
		*Thioglycollate Medium U.S.P.	CM173
		*Suitable for use in the techniques described in the British Pharmacopoeia or in the United States Pharmacopoeia.	
Microbial Limit Testing	– Coliforms	Brilliant Green Bile (2%) Broth	CM31
		Buffered Peptone Water	CM509
		Membrane Endo Agar LES	MM551
		MacConkey Broth (purple)	CM5a/CM6a
		Tergitol-7 Agar	CM793
		Violet Red Bile Agar	CM107
		Violet Red Bile Glucose Agar	CM485
	– Pseudomonas	Pseudomonas Agar Base	CM559
		Supplements	
		C-F-C Supplement	SR103
		or C-N Supplement	SR102
	– Salmonellae and Shigellae	Bismuth Sulphite Agar	CM201
		Brilliant Green Agar	CM263
		Desoxycholate Citrate Agar	CM35
		DCLS Agar	CM393
		Hektoen Enteric Agar	CM419
		Mannitol Selenite Broth Base	CM399
		Muller-Kauffmann Tetrathionate Broth Base	CM343
		Selenite Cystine Broth Base	CM699
	– Staphylococci	Baird-Parker Medium	CM275
		DNase Agar	CM321
		Giolitti-Cantoni Broth	CM523
		Mannitol Salt Agar	CM85
		Vogel-Johnson Agar	CM641
	– Enterococci	Azide Blood Agar Base	CM259
		Azide Dextrose Broth (Rothe Broth)	CM868
		Bile Aesculin Agar	CM888
		MacConkey Agar No.2	CM109
		Slanetz and Bartley Medium	CM377
		Kanamycin Aesculin Azide Agar Base	CM591

BREWING

The fermentation of hop-flavoured extracts of barley malt (wort) with 'top-fermenting' strains of *Saccharomyces cerevisciae* for English beers or 'bottom-fermenting' strains of *S. carlsbergensis* for Continental lagers, is a major industry in most parts of the world.

The most important concern of the brewing microbiologist is the establishment and maintenance of good plant hygiene. Infection of the brew with bacteria will cause 'off-flavours' and lead to considerable losses. Lowering the pH helps prevent infection by most bacteria but *Lactobacillus* and *Pediococcus* species are not affected and may still cause spoilage of the beer.

Of equal concern to the microbiologist is the quality and purity of the 'pitching' yeast i.e. the yeast inoculum used for the specific fermentation. Constant monitoring of the fermentation is required to detect the occurrence of 'wild' or non-specific yeasts which may appear during the brewing process. The fortunes of large brewing houses rest on the production of optically bright solutions of standardised colour and unvarying taste for what are, perhaps, the most critical consumers in the world. It follows, therefore, that every effort is made to control the brewing, filtration and bottling/canning stages of this most critical product.

MEDIA FOR BREWING

Organisms	Culture Media	Code
Coliforms	Lactose Broth	CM137
	MacConkey Agar	CM7
	MacConkey Broth (purple)	CM5a/CM6a
Lactobacilli	MRS Agar	CM361
	Rogosa Agar	CM627
	Tomato Juice Agar	CM113
	Raka-Ray Agar	CM777
Total contaminating bacteria (in yeast)	''Actidione'' Agar	PM118
Yeasts and Moulds	Lysine Medium ('wild' yeasts)	CM191
	Malt Extract Agar	CM59
	OGYE Agar	CM545
	Rose-Bengal Chloramphenicol Agar	CM549
	WL Nutrient Agar	CM309
	WL Nutrient Broth	CM501
	Wort Agar	CM247
	Yeast and Mould Agar	CM920

WATER SUPPLY AND SEWAGE DISPOSAL

The close connection between water fit for drinking and sewage disposal is best illustrated by the large towns which sit astride the major rivers in central USA. Each town draws water for consumption up-stream and discharges sewage effluent down-stream. The last town in such a chain may be drawing water containing the effluents of seven or eight large conurbations.

Such practices, which operate in all major countries, are safe, providing great care is taken in filtering and chlorinating the in-coming water. Equally, the processing of sewage must be safely operated so that pathogen-free and chemically clean effluent of low biological-oxygen-demand (BOD) is released back into the river down-stream.

Drinking water
Stored and river water may contain a wide variety of organisms, mainly saprophytic bacteria with optimal temperatures of growth around 22°C. Filtration and chlorination of the water, before distribution to the public, removes most of these organisms.

Microbiological tests are carried out to make sure that the quality of the treated water meets the specifications required by the Regulatory Authorities. *[DHSS 1982. The Bacteriological Examination of Drinking Water Supplies Report No.71. HMSO London. American Public Health Association. 1975. Standard Methods for the Examination of Water and Wastewater. 14th Edn. Washington D.C.]*

Bacterial pollution of water may originate from individuals with clinical symptoms of disease or from symptomless carriers of enteric pathogens such as *Salmonella typhi*. Such pathogens are difficult to detect in a water supply because their numbers are often few and their incidence sporadic. Therefore, indicator organisms of intestinal contamination are looked for because they are present in much larger numbers and they persist much longer than pathogens in polluted water.

From a public health point of view, the coliform test is the most important as the presence of *Escherichia coli* at >5 bacilli per 100ml of unchlorinated water indicates a less than satisfactory supply.

The quantitative assessment used is either a multiple tube, most probable number (MPN) or a membrane filtration method. The exact techniques and media used are cited in the references mentioned or in other national reference publications. *Clostridium perfringens* and *Enterococcus faecalis* can persist in water supplies for long periods. Their presence in water, when coliform organisms are absent, indicates faecal contamination at a more remote time.

Sewage disposal
In highly industrialised countries where large communities have developed, the disposal of industrial and domestic waste is an increasing problem.

International opinion is against untreated sewage being discharged into coastal or estuarine waters and the use of efficient treatment plants to process sewage before discharge is now recommended.

Untreated sewage consists mainly of water containing organic and inorganic dissolved and suspended substances, together with many micro-organisms. After preliminary screening to remove solid matter, the liquid is treated by one of three common methods:

(i) activated sludge process – this involves vigorous stirring or aeration by other means to reduce the BOD and cause separation of the organic matter.

(ii) biological filtration – in this process the liquid is filtered through large beds of sand and the micro-organisms are trapped in the zoogleal slime which forms during filtration.

(iii) oxidation ponds – settled sewage is held in ponds or lagoons for 30 days before the supernatant fluid is released.

All three processes utilise living organisms to reduce the BOD of the effluent to levels where it can be discharged into waterways without causing pollution.

LEGIONNAIRE'S DISEASE
This disease, caused by inhalation of large numbers of *Legionella* species, is essentially a water-borne infection. The infection starts in air-conditioning plants where large volumes of water are recirculated and cooled by blowing air through the water. Such warm circulated water quickly grows large quantities of legionellae and the aerosol of organisms caused by the air-cooling system spreads down-wind to infect passers-by. Not every passer-by develops the disease of legionellosis and the characteristics of susceptible victims are still being determined but a major factor is the quantity of organisms inhaled. A large inhaled dose of legionellae will inevitably lead to atypical pneumonia.

The most severe form of the disease is caused by *Legionella pneumophila* SG1 and it can be rapidly fatal without prompt antimicrobial treatment.

The organisms can easily be isolated from the water using specific legionella media as described in this manual. Isolation of the organism from the patient is more difficult and most infections are diagnosed by immunological tests.

It is now advised that all recirculating, air-cooled water systems are treated at regular and frequent intervals with bactericidal compounds to prevent the build-up of large numbers of legionellae.

MEDIA FOR WATER AND SEWAGE MICROBIOLOGY

Groups of Micro-organisms/Test	Purpose	Media	Code
Clostridium perfringens	Detection of *Clostridium perfringens* indicating remote or intermittent pollution in water	Iron Sulphite Agar	CM79
		Perfringens Agar (OPSP)	CM543
		Supplement A	SR76
		Supplement B	SR77
		Perfringens Agar Base TSC/SFP	CM587
		TSC Supplement	SR88
		SFP Supplement	SR93
		Reinforced Clostridial Medium	CM149
		Reinforced Clostridial Agar	CM151
	Confirmation of *Clostridium perfringens*		
	(a) by 'stormy-clot' reaction	Crossley Milk Medium	CM213
	(b) by Nagler Test	Blood Agar Base	CM55
	(c) Reverse CAMP Test	Egg Yolk Emulsion	SR47
		Fildes Extract	SR46
Coliform Group (including Esch. coli)	Detection of coliforms or *Esch. coli* (at 44°C) as indicators of pollution		
	(a) Mean Probable Number Technique	Lactose Broth	CM137
		Lauryl Tryptose Broth	CM451
		MacConkey Broth (Purple)	CM5a
		Minerals Modified Medium Base	CM607
		Sodium Glutamate	L124
	(b) Membrane Filtration Technique	Membrane Media Dehydrated M-Endo Agar LES	MM551
		Basic Fuchsin Indicator	BR50
		Membrane Lauryl Sulphate Broth	MM615
		Tryptone Bile Agar	CM595
	Confirmation of coliforms or *Esch. coli*	Brilliant Green Bile (2%) Broth	CM31
		Endo Agar Base	CM479
		Basic Fuchsin Indicator	BR50
		MacConkey Agar	CM7
		MacConkey Agar No.3	CM115
		MUG Supplement	BR71
		Tergitol-7 Agar	CM793
		Violet Red Bile Lactose Agar	CM107
		Violet Red Bile Glucose Agar	CM485
	Detection of *Esch. coli* O157: H7	Sorbitol MacConkey Agar	CM813
		Eosin Methylene Blue Agar	CM69
		Escherichia coli O157 Latex Test A latex agglutination test for the identification of *Esch. coli* serogroup O157	DR620
	Differentiation of coliforms		
	(a) Methyl Red and Voges-Proskauer Tests	MRVP Medium	CM43
	(b) Citrate Utilisation	Simmons Citrate Agar	CM155
	(c) lndole Production	Tryptone Water	CM87

Groups of Micro-organisms/Test	Purpose	Media	Code
Enterococci	Detection and isolation of faecal streptococci from sewage and water	Azide Blood Agar Base	CM259
		Azide Dextrose Broth (Rothe Broth)	CM868
		MacConkey Agar No.2	CM109
		Slanetz and Bartley Medium (Enterococcus Agar)	CM377
		Kanamycin Aesculin Azide Agar Base	CM591
		K-F Streptococcus Agar	CM701
Legionellae	Isolation and identification of *Legionella* species	Legionella Media	
		For MWY Medium	
		Legionella CYE Agar Base	CM655
		Legionella MWY Selective Supplement	SR118
		For Edelstein BCYE Medium	
		Legionella CYE Agar Base	CM655
		Legionella BCYE Supplement	SR110
		and either	
		Legionella BMPA-a Selective Supplement	SR111
		or Legionella (GVPC) Selective Supplement	SR152
	Latex ID Test	L. Pneumophila serogroup 1	DR801
		L. Pneumophila serogroup 2–14	DR802
		Legionella species test reagent	DR803
		Nutrient Gelatin	CM135a
Plate Count	Colony count for indicating the efficiency of water treatment processes, or suitability of water for food preparation. Nutrient gelatin is used for the plate count of psychrophilic organisms e.g. *Pseudomonas* species and for testing gelatinase activity	R2A Agar	CM906
		Standard Plate Count Agar (APHA)	CM463
		Tryptone Soya Agar	CM131
		Tryptone Soya Broth	CM132
		Yeast Extract Agar	CM19
		Buffered Peptone Water	CM509
Salmonellae and Shigellae	Pre-enrichment procedures prior to isolation	Mannitol Selenite Broth Base	CM399
		Selenite Broth Base	CM395
	Enrichment procedures prior to isolation	Selenite Cystine Broth Base	CM699
		Sodium Biselenite	L121
		Tetrathionate Broth Base	CM29
		Tetrathionate Broth USA	CM671
		Rappaport Vassiliadis (RV) Enrichment Broth	CM669
		Rappaport Vassiliadis Soya (RVS) Peptone Broth	CM866
		Bismuth Sulphite Agar	CM201
	Isolation and identification of *Salmonella* and *Shigella* species	Brilliant Green Agar (modified) with Salmonella Sulphamandelate Supplement	CM329/SR87
		DCLS Agar	CM393
		Desoxycholate Citrate Agar (Hynes)	CM227
		Hektoen Enteric Agar	CM419
		Kligler Iron Agar	CM33
		Lysine Decarboxylase Broth	CM308
		Lysine Iron Agar	CM381
		MLCB Agar	CM783
		Triple Sugar Iron Agar	CM277
		XLD Medium	CM469

Groups of Micro-organisms/Test	Purpose	Media	Code
Vibrios	Isolation and identification of the *Vibrionaceae*	Cholera Medium (TCBS)	CM333
		Aeromonas Medium Base (Ryan)	CM833
		Supplement – Ampicillin Supplement	SR136
		For presumptive identification of *vibrio* species	
		0129 Discs – 10µg per disc	DD14
		0129 Discs – 150µg per disc	DD15
Diluents used in water and sewage bacteriology	For preparation of 1/4 strength Ringer Solution, used as a diluent or rinse	Ringer Solution (1/4 strength Ringer Solution tablets)	BR52
	Preparation of a sodium hexametaphosphate Ringer Solution, used as a solvent diluent solution in conjunction with calcium alginate swabs	'Calgon' Ringer Tablets	BR49
	Preparation of chlorine-neutralising Ringer Solution, used as a diluent or rinse after hypochlorites or other chlorine sources	Thiosulphate Ringer Tablets	BR48

OXOID PRODUCTS FOR SPECIFIC GROUPS OF MICRO-ORGANISMS

Blood Agar Base Media
Blood Agar Base CM55
Blood Agar Base No.2 CM271
Blood Agar Base (sheep) CM854
Columbia Agar Base CM331
Tryptone Soya Agar CM131
Tryptose Blood Agar Base CM233

Organism
Actinomycetes:
Nocardia species
Streptomyces species

Isolation/Cultivation
Brain Heart Infusion Agar/Broth CM375/CM225
Czapek-Dox Agar CM97
Sabouraud Dextrose/Maltose Agar CM41/CM41a
Tryptone Soya Agar/Broth CM131/CM129
Thioglycollate Broth CM173

Differentiation
Nutrient Gelatin CM135a

Organism
Anaerobes:
Actinomyces species
Bacteroides species
Clostridium species
Fusiformis species
Streptococcus species

Isolation/Cultivation
Amies Transport Medium CM425
Blood Agar Base Media: see above
Brain Heart Infusion Agar/Broth CM375/CM225
Brucella Agar Base (USA) CM691
Clausen Medium CM353
Clostridium difficile Agar Base CM601
+ supplement SR96
Cooked Meat Medium CM81
Iron Sulphite Agar CM79
Liver Broth CM77
Nutrient Broth CM67
Perfringens Agar Base OPSP CM543
+ supplements SR76/SR77
Perfringens Agar Base TSC/SFP CM587
+ supplements SR88/SR93
Reinforced Clostridial Agar/Broth CM151/CM149
Schaedler Anaerobe Agar/Broth CM437/CM497
Thioglycollate Medium (Brewer) CM23
Thioglycollate Medium (USP) CM173
Thioglycollate Broth (USP Alternative) CM391
Tryptone Soya Agar/Broth CM131/CM129
Wilkens-Chalgren Anaerobe Agar/Broth CM619/CM643 + supplements SR107/SR108
Anaerobic Basal Agar CM972
Anaerobic Basal Broth CM957

Differentiation
An-Ident Discs DD6
Egg Yolk Emulsion SR47
Cooked Meat Medium CM81
Fildes Extract SR46
Iron Sulphite Agar CM79
Crossley Milk Medium CM213
Liver Broth CM77
SPS Discs DD16
Tryptone Water CM87
Wilkens-Chalgren Anaerobe Agar CM619/CM643 + supplements SR107/SR108
Enterotoxin Detection PET-RPLA TD900

Organism
Bacillus species

Isolation/Cultivation
Bacillus cereus Selective Agar Base CM617 + supplements SR99/SR47
Dextrose Tryptone Agar/Broth CM75/CM73
Nutrient Agar/Broth CM17/CM15
Tryptone Soya Agar/Broth CM131/CM129

Differentiation
Bacillus cereus Selective Agar Base CM617 + supplements SR99/SR47
Crossley Milk Medium CM2l3
Egg Yolk Emulsion SR47
Enterotoxin Detection BCET-RPLA TD950

Organism
Bordetella species

Isolation/Cultivation
Charcoal Agar CM119
+ supplement SR82
'Chocolate' Agar CM271

Differentiation
X and V factor discs DD3-DD5 on Blood Agar Base CM55

Organism
Brucella species

Isolation/Cultivation
Blood Agar Base No.2 CM271
Columbia Blood Agar Base CM331
Brucella Medium Base CM169
+ supplements SR83/SR35
Liver Broth CM77
Tryptone Soya Agar/Broth CM131/CM129

Differentiation
Brucella Medium Base CM169 with fuchsin and thionin dyes

Organism
Campylobacter species

Isolation/Differentiation
Bases:
Blood Agar Base No.2 CM271
Blood Free Campylobacter Selective Agar Base CM739
Campylobacter Agar Base CM689
Columbia Agar CM331

Supplements:
Blaser-Wang supplement SR98
Butzler supplement SR85

Growth supplement SR84
Skirrow supplement SR69
Preston supplement SR117
CCDA Selective supplement SR155
Campylobacter Selective supplement (Karmali) SR167
CAT supplement SR174
Laked Horse Blood SR48

Organism
Coli-Enterobacter Group

Resuscitation Media
Buffered Peptone Water CM509
E.E. Broth CM3l7
Maximum Recovery Diluent CM733
Membrane Lauryl Sulphate Broth MM615

Isolation/Cultivation
Blood Agar Base Media: see above
Brilliant Green Bile 2% Broth CM31
Cary-Blair Transport Medium CM519
China Blue Lactose Agar CM209
C.L.E.D. Medium CM301/CM423
Cooked Meat Medium CM81
Desoxycholate Agar CM163
Endo Agar Base CM479 + BR50
M-Endo Agar LES MM551 + BR50
Eosin Methylene Blue Agar (Levine) CM69
Lactose Broth CM137
Lauryl Tryptose Broth (Lauryl Sulphate Broth) CM451
MacConkey Agar CM7/CM7b/CM109/CM115
Sorbitol MacConkey Agar CM813
MacConkey Broth CM5
MacConkey Broth Purple CM5a
Minerals Modified Glutamate Medium Base CM607 + L124
Nutrient Agar/Broth CM3/CM1
Plate Count Agar CM325/CM463/CM681
Sorbitol MacConkey Agar CM813
Tergitol-7 Agar CM793
Tryptone Soya Agar/Broth CM131/CM129
Violet Red Bile Lactose Agar CM107
Violet Red Bile Glucose Agar CM485

Differentiation
Brilliant Green Bile 2% Broth CM31
Endo Agar Base CM479 + BR50
M-Endo Agar LES MM551 + BR50
Eosin Methylene Blue Agar (Levine) CM69
Esch. coli O157 Latex Test DR620
Lauryl Tryptose Broth CM451
MRVP Medium CM43
MUG Supplement BR71
Nutrient Gelatin CM135a
ONPG Discs DD13
SIM Medium CM435
Simmons Citrate Agar CM155
Tergitol-7 Agar CM793
Tryptone Bile Agar CM595
Tryptone Water CM87
Latex Test DR620

Organism
Corynebacterium species

Isolation/Cultivation
Blood Agar Base Media: see above
Hoyle Medium Base CM83 + SR30
Nutrient Broth No.2 CM67
Tinsdale Agar Base CM487 + SR65
Tryptone Soya Agar/Broth CM131/CM129

Differentiation
Blood Agar Base Media: see above
Hoyle Medium Base CM83 + SR30
Tinsdale Agar Base CM487 + SR65

Organism
Gardnerella species

Isolation/Differentiation
Columbia Agar Base CM331
+ supplement SR119
G. vaginalis Discs DD8/DD11

Organism
Haemophilus species

Isolation/Cultivation
Blood Agar Base Media: see above
Charcoal Agar CM119 + SR50
'Chocolate' Columbia Agar Base CM331 + SR50
Fildes Peptic Blood Agar – Blood Agar Base + SR46
Haemophilus Test Medium (HTM) Base CM898 + SR158

Differentiation
Charcoal Agar CM119
X, V, X+V factor discs DD3-DD5 placed on Blood Agar Base CM55
Identification beta-lactamase sticks BR66
Broad-Spectrum beta-lactamase Mixture SR113
Nitrocefin reagent SR112

Organism
Helicobacter pylori

Isolation/Cultivation
H. pylori Selective Medium
Columbia Blood Agar Base CM331
H. pylori Selective Supplement (Dent) SR147
H. pylori Kit DR720

Organism
Lactobacillus and *Leuconostoc* species

Isolation/Cultivation
L-S Differential Medium CM495
M17 Agar CM785
Milk Agar CM21
MRS Agar CM361
Orange Serum Agar CM657
Plate Count Agar CM325
Raka-Ray Agar CM777
Reinforced Clostridial Agar CM151
Reinforced Clostridial Broth CM149
Rogosa Agar CM627
Schaedler Anaerobe Agar CM437
Tomato Juice Agar CM113
Tryptone Soya Agar/Broth CM131/CM129

Differentiation
MRS Broth CM359
Tomato Juice Agar CM113

Organism
Legionella species

Isolation/Differentiation
Legionella CYE Agar Base CM655 with BCYE Growth Supplement SR110
+ BMPA-α Supplement SR111
or + MWY Supplement SR118
or + GVPC Supplement SR152
Legionella Latex Test DR800
L. Pneumophila serogroup 1 DR801
L. Pneumophila serogroup 2–14 DR802
Legionella species test reagent DR803

Organism
Listeria species

Isolation/Cultivation
Blood Agar Base Media: see above
Brain Heart Infusion Agar CM375
Buffered Listeria Enrichment Broth CM897
Listeria Selective Enrichment Supplement SR141
Fraser Broth CM895
Fraser Supplement SR156
Half-Fraser Supplement SR166
Listeria Selective Agar Base (Oxford) CM856
Listeria Selective Supplement SR140
Listeria Enrichment Broth CM862
Listeria Selective Enrichment Supplement SR141
Listeria Selective Enrichment Supplement (modified) with 10mg/l Acriflavine SR149
Listeria Enrichment Broth Base (UVM formulation) CM863
Listeria Primary Selective Enrichment Supplement (UVM) SR142
Listeria Secondary Selective Enrichment Supplement (UVM II) SR143
Listeria Rapid Test FT401
PALCAM Agar Base CM877
PALCAM Selective Supplement SR150

Organism
Mycobacterium species

Isolation/Differentiation
Acid Egg Medium PM1a
Lowenstein-Jensen Medium PM1 or PM2
Pyruvic Acid Egg Medium PM2a
Modified Acid Egg Medium PM95
Modified Pyruvic Acid Egg Medium PM96
Simplified Pyruvate Loewenstein-Jensen Medium PM98
Standard Reference Acid Egg Medium PM99
Standard Reference Pyruvic Acid Egg Medium PM100
Simplified Lowenstein-Jensen Medium PM97

Organism
Mycoplasma species

Isolation/Differentiation
Blood Agar Base Media: see above
Mycoplasma Agar Base CM401 + supplement SR59 or SR60
Mycoplasma Broth Base CM403 + supplement SR59 or SR60

Organism
Neisseria species

Isolation/Cultivation
Blood Agar Base Media: see above
Charcoal Agar CM119 + SR50
'Chocolate' Columbia Agar Base CM331 + SR50
GC Agar Base CM367 with Haemoglobin Powder Soluble L53 and Growth Supplements Yeast Autolysate SR105, Vitox SR90
+ GC supplement SR56
or + VCNT supplement SR91
or + VCN supplement SR101
or + LCAT supplement SR95
or + VCAT supplement SR104
Stuart Transport Medium (Modified) CM111

Differentiation
Identification beta-lactamase Sticks BR66
Identification oxidase Sticks BR64
Broad-Spectrum beta-lactamase Mixture SR113
Nitrocefin reagent SR112

Organism
Pasteurella/Yersinia species

Isolation/Cultivation
Blood Agar Base Media: see above
Cary Blair Transport Medium CM519
Desoxycholate Citrate Agar (Hynes) CM227
MacConkey Agar CM7b
SS Agar CM99
Yersinia Selective Agar Base CM653 + supplement SR109

Differentiation
Kligler Iron Agar CM33
Triple Sugar Iron Agar CM277
Urea Agar Base/Broth Base CM53/CM71 + SR20

Organism
Pseudomonas species

Isolation/Cultivation
Blood Agar Base CM55
Pseudomonas Agar Base CM559 + CFC supplement SR103
or + CN supplement SR102

Differentiation
Oxidase Sticks BR64
MacConkey Agar CM7b

Organism
Salmonella and *Shigella* species
Enrichment Media
Buffered Peptone Water CM509
EE Broth CM317
Lactose Broth CM137
Mannitol Selenite Broth Base CM399
Maximum Recovery Diluent CM733
Mueller-Kauffmann Tetrathionate Broth CM343
Rappaport Vassiliadis (RV) Broth CM669
Rappaport Vassiliadis Soya Peptone (RVS) Broth CM866
Selenite Broth Base CM395 + L121
Selenite Cystine Broth Base CM699
Tetrathionate Broth Base CM29
Tetrathionate Broth (USA) CM671

Isolation Media
Bismuth Sulphite Agar (Modified) CM201
Brilliant Green Agar CM263

Brilliant Green Agar (Modified) CM329 + Sulphamandelate supplement SR87
DCLS Agar CM393
Desoxycholate Citrate Agar CM35
Desoxycholate Citrate Agar (Hynes) CM227
Endo Agar Base CM479 + BR50
Hektoen Enteric Agar CM419
MacConkey Agar CM115
M-Endo Agar LES MM551 + BR50
MLCB Agar CM783
SS Agar CM99
SS Agar (Modified) CM533
XLD Medium CM469

Differentiation
Kligler Iron Agar CM33
Lysine Decarboxylase Broth CM308
Lysine Iron Agar CM381
MR-VP Medium CM43
Simmons Citrate Agar CM155
Triple Sugar Iron Agar CM277
Tryptone Water CM87
Urea Agar Base/Broth Base CM53/CM71 + SR20
Salmonella Rapid Test:
Elective Medium CM857
Rapid Test FT201
Latex Test FT203

Organism
Staphylococcus species

Isolation/Cultivation
Baird-Parker Medium CM275 + SR47/SR30 or SR54 *or* + RPF supplement SR122
Blood Agar Base Media: see above
China Blue Lactose Agar CM209
Columbia Agar Base CM331 + Staph/Strep supplement SR70
Giolitti-Cantoni Broth CM523
MacConkey Agar CM7b
Mannitol Salt Agar CM85
Nutrient Broth CM67
Salt Meat Broth CM94
Staphylococcus Medium No.110 CM145
Vogel-Johnson Medium CM641

Differentiation
Baird-Parker Medium CM275 + SR47/SR30 or SR54 *or* + RPF supplement SR122
Beta-Lactamase Sticks BR66
DNase Agar CM321
Egg Yolk Emulsion SR47
Egg Yolk Tellurite Emulsion SR54
Mannitol Salt Agar CM85
Nitrocefin SR112
Nutrient Gelatin CM135a
Staphylococcus Medium No.110 CM145
Staphylase Test DR595
Staphytect DR650

Organism
Streptococcus and *Enterococcus* species

Isolation/Cultivation
Amies Transport Medium CM425
Azide Blood Agar Base CM259
Azide Dextrose Broth CM868
Blood Agar Base Media: see above + Streptococcus supplement SR126
Brain Heart Infusion Agar/Broth CM375/CM225
China Blue Lactose Agar CM209
Dextrose Broth CM175
Dextrose Tryptone Agar/Broth CM75/CM73
Edwards Medium (Modified) CM27
GBS Agar Base (Islam) CM755 + SR35
Kanamycin Aesculin Azide Agar Base CM591 + supplement SR92
Kanamycin Aesculin Azide Broth Base CM771 + supplement SR92
KF Streptococcus Agar CM701
MacConkey Agar No.2 CM109
MacConkey Broth CM5
M17 Agar CM785
Nutrient Broth CM67
Slanetz and Bartley Medium CM377
Stuart Transport Medium (Modified) CM111
Todd-Hewitt Broth CM189
Tryptone Soya Agar/Broth CM131/CM129

Differentiation
Azide Blood Agar CM259
Bacitracin Discs DD2
Edwards Medium (Modified) CM27
KAA Agar Base/Broth Base CM591/CM771 + supplement SR92

Nutrient Gelatin CM135a
Optochin Discs DD1
Streptococcal Grouping Kit DR585

Organism
Vibrionaceae:
Aeromonas species
Plesiomonas species
Vibrio species

Isolation/Cultivation
Aeromonas Medium Base (Ryan) CM833 + supplement SR136
Blood Agar Base Media: see above
Cary Blair Transport Medium CM519
Cholera Medium TCBS CM333
DCLS Agar CM393
Peptone Water CM9
Tryptone Soya Agar/Broth CM131/CM129

Differentiation
Cholera Medium TCBS CM333
Identification Oxidase Sticks BR64
MR-VP Medium CM43
Nutrient Gelatin CM135a
0129 Discs DD14/DD15
Peptone Water CM9
Simmons Citrate Agar CM155
Tryptone Water CM87

Organism
Yeasts and Moulds

Isolation/Cultivation
'Actidione' Agar PM118
Brain Heart Infusion Agar CM375
Corn Meal Agar CM103
Czapek Dox Agar (Modified) CM97
Dermasel Agar CM539 + SR75
Dichloran-Glycerol (DG18) Agar Base CM729 + SR78
DRBC Agar Base CM727 + SR78

Eosin Methylene Blue Agar (Levine) CM69
Lysine Medium CM191
Malt Extract Agar/Broth CM59/CM57
OGYE Agar Base CM545 + SR73
Potato Dextrose Agar CM139
Rose-Bengal Chloramphenicol Agar CM549 + SR78
Sabouraud Media CM41/CM41a/CM147
Tryptone Soya Agar/Broth CM131/CM129
WL Nutrient Agar/Broth CM309/CM501
Wort Agar CM247
Yeast and Mould Agar CM920

Differentiation

Corn Meal Agar CM103
Czapek Dox Agar (Modified) CM97
Dermasel Agar CM539 + SR75
Eosin Methylene Blue Agar (Levine) CM69
Lysine Medium CM191
Sabouraud Media CM41/CM41a
WL Nutrient Agar CM309

CULTURE MEDIA PRODUCT DESCRIPTIONS

AEROMONAS MEDIUM BASE (RYAN)

Code: CM833

A selective diagnostic medium for the isolation of Aeromonas hydrophila from clinical and environmental specimens when used with Ampicillin Selective Supplement SR136.

Formula	gm/litre
Proteose peptone	5.0
Yeast extract	3.0
L. Lysine monohydrochloride	3.5
L. Arginine monohydrochloride	2.0
Sorbitol	3.0
Inositol	2.5
Lactose	1.5
Xylose	3.75
Bile Salts No.3	3.0
Sodium thiosulphate	10.67
Sodium chloride	5.0
Ferric ammonium citrate	0.8
Bromothymol blue	0.04
Thymol blue	0.04
Agar	12.5
Final pH 8.0 $\pm$ 0.1	

AMPICILLIN SELECTIVE SUPPLEMENT

Code: SR136
Vial contents (each vial is sufficient for 500ml of medium)

Ampicillin	2.5mg

Directions
Suspend 29.5g of Aeromonas Agar Base (Ryan) in 500ml of distilled water. Bring gently to the boil. DO NOT AUTOCLAVE. Cool to 50°C and aseptically add one vial of rehydrated Ampicillin Selective Supplement SR136 as directed. Mix well and pour plates.

Description
Ryan[1] modified the formulation of XLD Medium so that it would support the growth of *Aeromonas* spp and *Plesiomonas* spp as well as the usual enterobacteriaceae. It could therefore be used as a universal medium in the investigation of enteric disease. However, to improve its performance in the isolation of aeromonads, the addition of ampicillin at 5mg/l is recommended. The effectiveness of ampicillin as a selective agent for *Aeromonas* spp has been reported by several workers[2,3,4,5,6]. The value of Aeromonas Medium Base (Ryan) is that the recommended level of ampicillin is well below that which can cause inhibition of some strains of aeromonads.[7]

The utility of Aeromonas Medium (Ryan) and its superiority over some other formulae for detection of *Aeromonas* species in tap water, bottled water and foods including meat, poultry, fish and seafoods has been reported[8,9,10]. Aeromonas Medium (Ryan) is specified by the MAFF/DHS Steering Group on the Microbiological Safety of Food for detection and enumeration of *Aeromonas hydrophila* in clinical specimens.[11] *Aeromonas* spp occur widely in soil and water, where they cause diseases in fish and amphibians. They also occur in untreated and chlorinated drinking water, raw foods and raw milk.[11,12] It is considered that the major cause of gastrointestinal infections by *Aeromonas* spp[12,13] is from ingesting infected water.[14,15]

The role of these organisms in gastrointestinal disease is still subject to debate but a rapidly expanding body of literature suggests that *Aeromonas* spp can cause a wide spectrum of enteric symptoms in adults as well as children.[5,16] It would therefore be a useful diagnostic aid to include this selective medium when investigating diarrhoeal disease.

Oxoid Aeromonas Medium Base has been developed to improve the enumeration and isolation of *Aeromonas* spp from clinical and environmental specimens.

Technique
1 Prepare the medium according to directions and pour into sterile dishes. The prepared medium may be stored at 2–8°C up to 5 days.
2 Inoculate the plates with a suspension of food, faeces etc., diluted to form single colonies on the inoculated plate.
3 Incubate the plates aerobically at 30–35°C for 24 hours. If further incubation is required hold at room temperature (22–25°C).
4 Examine the plates for the presence of dark green, opaque colonies with darker centres. Confirm the identity with biochemical tests.

The typical colonial appearance of *Aeromonas* isolates on this medium is as follows:

Aeromonas species:
Dark green, opaque with darker centre, diameter 0.5–1.5mm.

Pseudomonas species:
Blue/grey translucent, diameter from pinpoint to 0.25mm.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium 2–8°C.

Quality Control
Positive control:
Aeromonas hydrophila ATCC® 7966

Negative control:
Escherichia coli ATCC® 25922

Precautions
Although *Aeromonas* and *Plesiomonas* spp will grow on the medium if ampicillin is omitted, it will be more difficult to distinguish them from the other organisms present on the plate. Suspected colonies of *Aeromonas* spp must be confirmed by biochemical tests.

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AMIES TRANSPORT MEDIUM

Code: CM425

An improved transport medium, containing charcoal to prolong the viability of pathogenic organisms.

Formula	gm/litre
Charcoal pharmaceutical	10.0
Sodium chloride	3.0
Sodium hydrogen phosphate	1.15
Potassium dihydrogen phosphate	0.2
Potassium chloride	0.2
Sodium thioglycollate	1.0
Calcium chloride	0.1
Magnesium chloride	0.1
Agar	4.0
pH 7.2 $\pm$ 0.2	

Directions
Suspend 20g in 1 litre of distilled water. Bring to the boil to dissolve the agar completely. Distribute into small, screwcap bottles, stirring meanwhile to keep the charcoal evenly suspended. Screw down the caps firmly on the completely filled bottles. Sterilise by autoclaving at 121°C for 15 minutes. Invert the bottles whilst cooling to distribute the charcoal uniformly. Store in a cool place.

Description
Amies[1] modified Stuart's Transport Medium[2,3,4] by replacing glycerophosphate with an inorganic phosphate buffer and adding charcoal to the medium.

The metabolism of glycerophosphate by coliform organisms and other Gram-negative rods in Stuart's original formulation resulted in the proliferation of these organisms from wound swabs and faecal specimens.

A concentration of NaCl at 0.3% w/v was discovered by Amies to be optimal for the preservation of *Neisseria gonorrhoeae.*

Calcium and magnesium salts were added in the belief that these ions were of importance in controlling the permeability of the bacterial cells and so contributing to their survival.

Stuart[3] showed that the survival of *N. gonorrhoeae* was increased by the use of charcoal swabs, but because they were black and dusty, they proved unpopular with the patients. Amies[1] overcame this problem by incorporating charcoal in this medium.

Survival of N. gonorrhoeae at 22°C

85 strains	***with Charcoal***	***without Charcoal***
Time	***No. of strains surviving***	
24 hours	82	20
48 hours	70	0
72 hours	38	0
100 strains	***in Stuart's Medium***	***in Amies Medium***
Time	***No. of strains surviving***	
24 hours	91	98
48 hours	79	87
72 hours	56	77

(Tables taken from Amies[1]).

The agar concentration was increased from that proposed by Stuart because the presence of charcoal particles interferes with the gelling properties of the agar.

Amies removed the methylene blue indicator from Stuart's formulation considering it superfluous because of the presence of charcoal in the medium. Care should be taken to ensure that the prepared bottles of transport medium are not stored longer than 9 months from the date of preparation, or freshly steamed and the charcoal resuspended before use.

The value of these modifications was shown in two studies which tested the efficiency of various transport media.[5,6]

Amies Transport Medium is recommended for the transport of specimens to be cultured for *Bacteroides ureolyticus.*[7]

Technique
Use sterile, cotton-tipped swabs on wooden sticks to collect the specimen. Push the swab down one third of the medium depth and cut the stick so that when the cap is screwed down, the swab is forced to the bottom of the medium.

Make sure the cap is screwed firmly on the bottle and keep cool during the transport period.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

The prepared medium, held in tightly screw-capped bottles, can be stored at room temperature.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923.
Escherichia coli ATCC® 25922

Negative control:
Uninoculated medium.

Precautions
It is important that the charcoal is properly suspended in the medium, invert the bottles when the bottles are cool but the agar still liquid.

During preparation of the medium, avoid prolonged heating in open flasks because thioglycollate is volatile.

Old medium should be freshly steamed and the charcoal resuspended before use.

Keep medium cool during transport but do not freeze.

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ANAEROBE BASAL AGAR

Code: CM972

A nutrient agar for the growth of anaerobic micro-organisms, particularly Bacteroides spp. and other fastidious anaerobes.

Formula	gm/litre
Peptone	16.0
Yeast extract	7.0
Sodium chloride	5.0
Starch	1.0
Dextrose	1.0
Sodium pyruvate	1.0
Arginine	1.0
Sodium succinate	0.5
Sodium bicarbonate	0.4
L-cysteine HCl	0.25
Ferric pyrophosphate	0.5
Haemin	0.005
Vitamin K	0.0005
Dithiothreitol	0.25
Agar	12.0
pH 7.0 ± 0.2	

Directions
Suspend 46g in 1 litre of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50–55°C and aseptically add 5–10% sterile Defibrinated Horse Blood SR50.

Mix well and pour in sterile petri dishes.

500g of this medium makes 10.8 litres.

Description
Anaerobe Basal Agar CM972 contains peptones, carefully selected to support good growth of anaerobic bacteria and yeast extract as a vitamin source. Starch is present to absorb any toxic metabolites[1]. Sufficient arginine is added to ensure the growth of *Eubacterium lentum*[2], whilst haemin and vitamin K are growth factors required by many *Bacteroides* species[3]. Haemin is also required by *Porphyromonas* species. Sodium succinate improves the growth of *Prevotella melaninogenica* and *Bacteroides* species[4]. Sodium pyruvate is added as an energy source for asaccharolytic cocci such as Veillonella. It also acts similarly to catalase and degrades traces of hydrogen peroxide, which may be produced by the action of molecular oxygen on media components[5]. L-cysteine hydrochloride and dithiothreitol are reducing agents, and cysteine has also been shown to stimulate the growth of some anaerobes[6].

Technique
Inoculate the medium by surface plating to obtain single colonies. Incubate anaerobically for up to 5 days at 37°C. Anaerobic conditions can be achieved using the Oxoid AnaeroGen Atmosphere Generation System AN025A with the Oxoid AnaeroJar AG025A.

The prepared medium may be stored for up to 3 weeks at 2–8°C in the dark.

The medium may be rendered selective for Gram-negative anaerobes by the addition of G-N supplement SR108 and for non-sporing anaerobes by the addition of N-S supplement SR107 with Tween 80. Neomycin Selective Supplement SR163 can be added to select for *Clostridia*.

Storage conditions and Shelf life
Anaerobe Basal Agar CM972 should be stored tightly capped in the original container at 10°C–25°C. When stored as directed, the medium will remain stable until the expiry date printed on the bottle.

Quality Control
Positive control:

Peptostreptococcus anaerobius	ATCC® 27337
Prevotella melaninogenicus	ATCC® 15930
Clostridium perfringens	ATCC® 13124

Negative control:
Uninoculated medium.

Precautions
Anaerobe Basal Agar CM972 should be used for *in vitro* purposes only. Do not use beyond the stated expiry date, or if the product is caked, discoloured or shows any sign of deterioration.

References

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Anaerobe Basal Broth

Code: CM957

A nutrient broth for the growth of anaerobic micro-organisms, particularly Bacteroides spp. and other fastidious anaerobes.

Formula	gm/litre
Peptone	16.0
Yeast extract	7.0
Sodium chloride	5.0
Starch	1.0
Dextrose	1.0
Sodium pyruvate	1.0
Arginine	1.0
Sodium succinate	0.5
L-cysteine HCl	0.5
Sodium bicarbonate	0.4
Ferric pyrophosphate	0.5
Haemin	0.005
Vitamin K	0.0005
Sodium thioglycollate	0.5
Dithiothreitol	1.0

Directions
Suspend 35.4g of Anaerobe Basal Broth in 1 litre of distilled water. Bring to the boil to dissolve completely. Distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Oxoid Anaerobe Basal Broth CM957 is formulated from a range of nutrients which have been selected to optimise the recovery and growth of the majority of anaerobic organisms of clinical importance.

The formulation includes yeast extract as a source of vitamins and starch is included to absorb toxic products[1]. Sufficient arginine is added to ensure growth of *Eubacterium lentum*[2], whilst haemin and vitamin K are present as they are essential for the growth of *Bacteroides* spp[3]. Pyruvate is present as an energy source for asaccharolytic cocci such as *Veillonella* spp. It also eliminates traces of hydrogen peroxide which may be produced by the action of molecular oxygen on medium constituents[4]. Sodium succinate improves the growth of *Prevotella melaninogenica* and *Bacteroides* spp[5]. It is included together with the reducing agent L-cysteine hydrochloride, which has been shown to stimulate directly the growth of some anaerobes[6].

Technique
It is preferable to use freshly reconstituted and sterile medium which is inoculated as soon as it has cooled to room temperature. Tubes which are not used on the day of preparation should be placed in a boiling water bath or steamer for approximately 15 minutes to remove dissolved oxygen. They should be allowed to cool without agitation and then inoculated.

Storage conditions and Shelf life
Anaerobe Basal Broth CM957 should be stored tightly capped in the original container at 10°C–25°C. When stored as directed, the medium will remain stable until the expiry date printed on the bottle.

Quality Control
Positive control:

Peptostreptococcus anaerobius	ATCC® 27337
Prevotella melaninogenica	NCTC 11321
Clostridium perfringens	ATCC® 13124

Negative control:
Uninoculated medium

Precautions
Anaerobe Basal Broth CM957 should only be used for *in vitro* diagnostic purposes.

Do not use beyond the stated expiry date, or if the product is caked, discoloured or shows any sign of deterioration.

References
1 Ajello GW. Geely JC. Hayes PS. *et al.* Trans-isolate medium: a new medium for primary culturing and transport of *Neisseria meningitidis, Streptococcus pneumoniae* and *Haemophilus influenzae*. J. Clin. Micro. 1984:20:55–8.
2 Sperry JF. Wilkins TD. Arginine, a growth-limiting factor for *Eubacterium lentum*. J. Bacteriol 1976:127:780–4.
3 Gibbons RJ. and MacDonnald JB. Haemin and vitamin K compounds as required factors for the cultivation of certain strains of *Bacteroides melaninogenicus*. J. Bact. 1960:80:164–170.
4 Neilson PA. Role of reduced sulphur compounds in nutrition of *Proprionobacterium acnes*. J. Clin. Micr. 1983:17:276–9.
5 Lev M. Keudell KC. and Milford AF. Succinate as a growth factor for *Bacteroides melaninogenicus*. J. Bact. 1971:108:175–8.
6 Shanson DC. and Singh J. Effect of adding cysteine to brain-heart infusion broth on the isolation of *Bacteroides fragilis* from experimental blood cultures. J. Clin. Path. 1981:34:221–3.

Antibiotic Assay Media

Codes: CM327/CM335/CM287

The methods and media required for the bio-assay of antibiotics in pharmaceutical products, foods and other materials are specified in the US Pharmacopoeia XX[1] and by the FDA[2] as well as other works of reference[3]. Grove and Randall[4] defined the media formulations which are best for the different assay organisms used when testing for different antibiotics and these are numbered 1 to 13, 19, 20 and 21. To avoid confusion the original numbers given by Grove and Randall are retained but some of the formulations are less widely used than others.

Antibiotic media Nos. 1, 2 and 3 are the most widely used and there is the advantage that antibiotic media Nos. 5, 8 and 11 can be prepared from them by simple pH adjustment.

pH ranges of Antibiotic Assay Media

5.6–5.7	6.6	7.0	7.9–8.0
No. 8	Nos. 1 & 2	No. 3	Nos. 5–11

The assay of antibiotics is a highly skilled process which requires very close attention to the details specified in the official publications and these must be consulted. The general principles are, however, straightforward and can be divided into (1) diffusion plate assay and (2) turbidometric assay.

Diffusion plate assays depend upon the diffusion of antibiotics from reservoirs on or in the agar layer. Measurement of the amount of diffusion is made by comparing the sizes of zones of growth inhibition, using standard strains of susceptible organisms and known amounts of reference antibiotics.

The results depend on critical rates of diffusion of the antibiotic, critical growth rates of the standard organisms and critical minimal inhibitory coefficient levels of each organism.

The reservoirs may be holes cut into the agar and filled with known concentrations of antibiotic, or metal cylinders placed on the surface of the agar and similarly filled, or paper discs charged with antibiotic.

Large square plates (250 x 250mm) or flat sheets of glass are commonly used and a 4mm depth of base agar is poured and allowed to set. Then a thin (1mm) layer of seeded agar is poured over the base layer and allowed to set. The holes are punched into these layers or the various reservoirs placed on the seed layer. It is essential that the layers of agar are even in depth and care must be taken to pour them on properly levelled benches. Up to 36 separate reservoirs can be placed in dishes of this size.

ANTIBIOTIC MEDIA USED FOR MICROBIOLOGICAL ASSAYS

Antibiotic	Organism	Inoc.	Diffusion		Turbidomet.
			Base	Seed	
Amikacin	*Staph. aureus* ATCC® 6538P	1			3
Amoxicillin	*Micro. luteus* ATCC® 9341	1	11	11	
Ampicillin	*Micro. luteus* ATCC® 9341	1	11	11	
Cephalosporins	*Staph. aureus* ATCC® 6538P	1	2	1	
Chloramphenicol	*Esch. coli* ATCC® 10536	1			3
Cloxacillin	*Staph. aureus* ATCC® 6538P	1	2	1	
Erythromycin	*Micro. luteus* ATCC® 9341	1	11	11	
Gentamycin	*Staph. epiderm.* ATCC® 12228	1	11	11	
Kanamycin	*Staph. aureus* ATCC® 6538P	1			3
Methicillin	*Staph. aureus* ATCC® 6538P	1	1	2	
Neomycin	*Staph. aureus* ATCC® 6538P	1	11	11	
Penicillin	*Staph. aureus* ATCC® 6538P	1	2	1	
Streptomycin	*Bac. subtilis* ATCC® 6633	1	5	5	
	Kleb. pneumo. ATCC® 10031	1			3
Tetracycline	*Staph. aureus* ATCC® 6538P	1			3
Tobramycin	*Staph. aureus* ATCC® 6538P	1			3
Vancomycin	*Bac. subtilis* ATCC® 6633	1	8	8	

ATCC® is a registered trade mark of the American Type Culture Collection.

Turbidimetric assays are an alternative method with advantages of short incubation times (3–4 hours) but they lack the accuracy of the diffusion assay[5]. Samples must be clear and not absorb the wavelength of light used in the spectrophotometer.

Dilutions of sample and dilutions of the antibiotic are made in parallel in 1ml volumes and 9ml volumes of seeded broth are added to each tube. The tubes are then incubated in a water bath for 3–4 hours at the appropriate temperature and at the end of the incubation period the growth is stopped by the addition of formaldehyde to each tube. The amount of growth that has taken place within the incubation period is measured by light transmission in a spectrophotometer. Comparison of the readings of the unknown sample and those of the known dilutions of the antibiotic will establish the level of antibiotic in the sample.

Standard organism cultures used in assays are normally maintained on agar slopes and transferred at 1–2 week intervals. The inoculum used for an assay is usually washed from an overnight culture on an agar slope, using saline or buffered peptone water and further diluted to a standard density. Spore suspensions of *Bacillus subtilis* and *B. cereus* are used and it is important to cultivate these organisms on media which enhance spore production e.g. Antibiotic Medium No.l with 300mg of manganese sulphate per litre added to the medium. Surface seed agar plates and examine the growth microscopically during the days of incubation until the majority of the organisms are seen to be bearing spores. Pasteurise the spore suspension to destroy the vegetative organisms and thus ensure clean edges to the zones of inhibition.

References

1 The United States Pharmacopeia, Biological Tests and Assays. XX. Revision, Rockville Md.: *United States Pharmacopeial Convention, 1980, pp. 882–888.*

2 Tests and Methods of Assay of Antibiotics and Antibiotic-Containing drugs, FDA, CFR, Title 21, Part 436, Subpart D, Washington DC: *US Government Printing Office, paras. 436.100–436.106, p. 242–259 (April 1) 1983.*

3 Hausler W. J. (Ed) *Standard Methods for the Examination of Dairy Products, 13th edn. Washington D.C.: American Public Health Association, 1972.*

4 Grove D.C. and Randall W.A. Assay Methods of Antibiotics, *New York: Medical Encyclopedia 1955.*

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ANTIBIOTIC MEDIUM NO. 1

SEED AGAR

Code: CM327

A medium recommended for the seed layer in the preparation of plates for the microbiological assay of antibiotics.

Formula	gm/litre
Peptone	6.0
Tryptone	4.0
Yeast extract	3.0
'Lab-Lemco' powder	1.5
Glucose	1.0
Agar No.1	11.5
pH 6.5 ± 0.2	

Directions
Suspend 27g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
This is perhaps the most important medium in antibiotic assay work and it is in a specially modified form to take advantage of the properties of Oxoid Agar No.1.

Hanus, Sands and Bennett[1] drew attention to the inhibitory properties which certain agars have towards some antibiotics, particularly streptomycin, kanamycin, polymyxin B and neomycin. Because Oxoid Agar No.1 does not share these inhibitory properties it is especially suited to antibiotic assay work. In addition, this agar, with its superior diffusion properties, produces more clearly defined inhibition zones.

NB *In the Oxoid formulation, only 11.5 grams of Agar No. 1 are used to give the equivalent gel strength of 15 grams ordinary agar.*

This medium is used as a seed agar with *Micrococcus flavus* for the plate assay of bacitracin; with *Sarcina lutea* for the plate assay of chloramphenicol and with *Staphylococcus aureus* for the assay of kanamycin sulphate, penicillin G, sodium methicillin and sodium oxacillin.

It is also employed as a base agar in the assay of the following drugs: chloramphenicol, kanamycin sulphate, colistin sulphate, sodium methicillin, sodium oxacillin and vancomycin hydrochloride.

Reference

1 Hanus F. J., Sands J. G. and Bennett E. O. (1967) *Applied Microbiology 15(1) 31–34.*

ANTIBIOTIC MEDIUM NO. 2

BASE AGAR

Code: CM335

A standard medium for use as a base layer in the preparation of plates for the microbiological assay of antibiotics.

Formula	gm/litre
Peptone	6.0
Yeast extract	3.0
'Lab-Lemco' powder	1.5
Agar No.1	10.0
pH 6.5 ± 0.2	

Directions
Suspend 20.5g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
This new formulation replaces the older product labelled 'Base Agar' which has been withdrawn.

Medium No.2 is used as the base agar in the plate assay of bacitracin and in the assay of penicillin G.

ANTIBIOTIC MEDIUM NO. 3

ASSAY BROTH

Code: CM287

Used in the serial dilution assay of penicillin and other antibiotics.

Formula	gm/litre
Peptone	5.0
Yeast extract	1.5
'Lab-Lemco' powder	1.5
Glucose	1.0
Sodium chloride	3.5
Dipotassium hydrogen phosphate	3.68
Potassium dihydrogen phosphate	1.32
pH 7.0 ± 0.2	

Directions
Add 17.5 grams to 1 litre of warm (60°C) distilled water and mix well to dissolve. Distribute and sterilise by autoclaving at 121°C for 15 minutes.

Description
Medium No.3 is used in the turbidimetric assay of penicillin and tetracycline with *Staphylococcus aureus*.

Taking advantage of modern technology, this medium is based on the original 'Penassay Broth' which has been withdrawn.

ANTIBIOTIC MEDIUM NO. 5

STREPTOMYCIN ASSAY AGAR

This is very easily made by changing the pH of Antibiotic Medium No.2. To one litre of Base Agar add 5ml of normal caustic soda solution. This may be carried out either prior to autoclaving or aseptically after sterilisation. This will bring the pH to 7.9, but no filtration will be necessary. This medium is suitable for the streptomycin assay, using the cylinder plate technique and a test organism of *Bacillus subtilis* and gives clearly defined inhibition zones.

After this base layer has gelled, it is overlaid with the same medium already inoculated with spores of the test organism.

ANTIBIOTIC MEDIUM NO. 8

FOR THE PLATE ASSAY OF TETRACYCLINE AND VANCOMYCIN

This can readily be made by a small adjustment of the pH of Medium No.2. To convert Medium No.2 into Medium No.8 aseptically add 1.25ml of N/1 HCl, after sterilisation, to the still molten Medium No.2 at 50–55°C. This will bring the pH down to 5.6–5.7. No filtration will be necessary. Medium No.8 is used for the base agar and seed agar for the plate assay of tetracycline. It is also used as the seed agar for vancomycin hydrochloride.

ANTIBIOTIC MEDIUM NO.11

FOR THE PLATE ASSAY OF NEOMYCIN SULPHATE

This can readily be made by a small change of pH of Medium No.l. To one litre of Base Agar add 5ml of normal caustic soda solution. This may be carried out either prior to autoclaving or aseptically after sterilisation. The pH will then be 7.9–8.0. No filtration will be necessary. Medium No. 11 is used as the base agar and seed agar for the plate assay of neomycin sulphate.

ARCOBACTER BROTH

Code: CM965

An enrichment broth for Arcobacter species.

Formula	gm/litre
Peptone	18.0
Yeast extract	1.0
Sodium chloride	5.0

Directions for use with CAT Supplement SR174
Dissolve 12g of Arcobacter Broth CM965 in 500ml of distilled water. Sterilise at 121°C for 15 minutes.

Allow to cool to 50°C and add one vial of CAT Selective Supplement SR174E reconstituted as directed.

Dispense into sterile containers.

pH 7.2 ± 0.2

Directions for use with CCDA Supplement (SR155)
Dissolve 12g of Arcobacter Broth CM965 in 500ml of distilled water. Sterilise at 121°C for 15 minutes. Allow to cool to 50°C and add one vial of CCDA Selective Supplement SR155 reconstituted as directed.

Dispense into sterile containers.

Incubate at 30°C aerobically for 24 hours.

Description
Oxoid Arcobacter Broth CM965 is intended for use with Cefoperazone, Amphotericin B, Teicoplanin (CAT) Selective Supplement SR174 as a selective enrichment broth for the growth of *Arcobacter* species and with the more selective CCDA SR155 for the selective enrichment of *Arcobacter butzleri*.

Peptones in the base medium are specifically designed to provide the ideal growth conditions for *Arcobacter* species. The incubation conditions and the absence of blood or charcoal supplements suppress the growth of *Campylobacter* species. Cefoperazone, Amphotericin B and Teicoplanin are added to suppress the growth of competing flora, but allow the growth of *Arcobacter* species. CCDA Selective Supplement SR155 is substituted for CAT to selectively isolate *Arcobacter butzleri*[1,2].

Arcobacters are micro aerophilic, Gram-negative rods, which were formerly classified as *Campylobacter*[3].

Four *Arcobacter* species have been identified: *A. butzleri, A. cryaerophilus, A. skirrowii* and *A. nitrofigilis*, all of which have a greater propensity to grow in air than *Campylobacter* spp.

A. butzleri, A. cryaerophilus, and *A. skirrowii* have been associated with disease in humans[4,5], and typically are isolated from faecal samples.

A. butzleri has been isolated from patients with bacteraemia, peritonitis, endocarditis and diarrhoea. Patients with *A. butzleri*-associated diarrhoea typically suffer from abdominal pain and nausea, fever, chills, vomitting and malaise, but the organism has also been implicated in an outbreak of recurrent abdominal cramps without diarrhoea[6]. The source of infection is usually contaminated water or sewage[6].

A. cryaerophilus group 1B has been isolated from patients with bacteraemia and diarrhoea[4,5], although it is a much less common human isolate than *A. butzleri*[6].

A. nitrofigilis has only been isolated from marsh grass to date, never from humans or animals. It is not thought to be clinically significant[7].

Storage conditions and Shelf life
Arcobacter Broth CM965 should be stored at room temperature. When stored as directed, the reagents remain stable until the expiry date shown on the label.

Quality Control
Positive control:
Arcobacter butzleri ATCC® 12481 – white/grey colonies

Negative control:
Escherichia coli ATCC® 25922 – inhibited

Precautions
Arcobacter Broth CM965 should only be used for *in vitro* diagnostic purposes.

References

1 Data on file.
2 Lammerding, A.M., Harris, J.E., Lior, D.L. et al. Presented at the 81st annual meeting of IAMFES (1994).
3 Vandamme, P., Falsen, E., Rossau, R., Hoste, B., Segers, P., Tytgat, R., De Ley, J. (1991). *Int. J. Syst. Bacteriol.* 41:88–103.
4 Kiehlbauch, J.A., Brenner, D.J., Nicholson, M.A., Baker, C.N., Patton, C.M., Steigerwalt, A.G., Wachsmuth, I.K. (1991). *J. Clin. Microbiol.* 29:376–385.
5 Vandamme, P., Vancanneyt, M., Pot, B., Mels, L., Hoste, B., Dewettinck, D., Vlaes, L., Van den Borre, C., Higgins, R., Hommer, J. (1992). *Int. J. Syst. Bacteriol.* 42:344–356.
6 Vandamme, P., Pugina, P., Benzi, G., Van Etterick, R., Vlaes, L., Kersters, K., Butzler, J., Lior, H., Lauwers, S. (1992). *J. Clin. Microbiol.* 30:2335–2337.
7 Atabay, H.I and Corry, J.E.L. (1998) *Int. J. Food Microbiol*, 41, 53–58.

AZIDE BLOOD AGAR BASE

Code: CM259

A selective medium for the detection and isolation of streptococci and staphylococci from faeces, sewage and other specimens. With added blood, the medium may be employed for the simultaneous determination of haemolytic reactions.

Formula	gm/litre
Tryptose	10.0
'Lab-Lemco' powder	3.0
Sodium chloride	5.0
Sodium azide	0.2
Agar	12.0
pH 7.2 ± 0.2	

Directions
Suspend 30g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. For azide blood agar, cool to 45–50°C and add 5% of sterile blood.

Description
A selective medium for the detection and isolation of streptococci from faeces, sewage, and other specimens containing a mixed flora. Azide Blood Agar Base is similar to the medium used by Edwards[1] for the isolation of mastitis streptococci. The sodium azide has a bacteriostatic effect on most Gram-negative organisms but permits growth of Gram-positive organisms such as streptococci and some strains of staphylococci. *Proteus* species are slightly more resistant than other Enterobacteriaceae but swarming is prevented (Snyder and Lichstein[2], Lichstein and Snyder[3]).

At the above concentration and pH, sodium azide exerts no appreciable effect on haemolysis so that the medium, with added blood, may be used for the simultaneous determination of haemolytic reactions.

Azide blood agar is recommended by the American Public Health Association[4] for the isolation of streptococci from cheese. The plates, inoculated with dilutions of emulsified cheese, are incubated at 35°C and representative colonies subcultured for subsequent identification.

There are variations in formula of Azide Blood Agar Base which have been recommended for different purposes:

1 Packer[5] increased the sodium azide concentration to 0.9g per litre and added 0.002g per litre crystal violet. The pH was also adjusted to 6.8 ± 0.1. This is a more selective medium for faecal streptococci in foods[6].
2 Packer[5] and Wood[7] used the above formulation with 5% blood and the crystal violet increased to 0.01g per litre, for the isolation of *Erysipelothrix rhusiopathiae* and *Streptococcus pneumoniae*.
3 Dale[8] and Bohm[9] recommended the addition of phenol (1.0 to 2.5g per litre) to Packer's formulation to isolate *E. rhusiopathiae*.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store prepared blood agar plates of medium at 2–8°C.

Quality Control
Positive control:
Enterococcus faecalis ATCC® 29212
Staphylococcus aureus ATCC® 25923

Negative control:
Proteus vulgaris ATCC® 13315
Escherichia coli ATCC® 25922

Precautions
Proteus and *Escherichia* species may not always be inhibited on the Edward's formulation.

Always use a light inoculum for best selective results.

Anaerobic incubation will enhance haemolytic reactions.

Haemolytic reactions will not be typical on Packer's modification of Azide Blood Agar Base. *Streptococcus lactis* will not grow on Packer's modification with 5% sheep blood.

Read the section on Hazard Precautions 2.7 for azide-containing media.

References

1 Edwards S. J. (1933) *J. Comp. Path. Therap. 46(4) 211–217.*
2 Snydar M. L. and Lichstein H. C. (1940) *J. Infect. Dis. 67(2) 113–115.*
3 Lichstein H. C. and Snyder M. L. (1941) *J. Bact. 42(5) 653–664.*
4 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. New York.*
5 Packer R. A. (1943) *J. Bact. 46. 343–349.*
6 Mossel D. A. A., Diepen H. M. J. van and De Bruin A. S. (1957) *J. Appl. Bact. 20(2) 265–272.*
7 Wood R. L. (1965) *Amer. J. Vet. Res. 26. 1303–1308.*
8 Dale C. N. (1940) *J. Bact. 40. 228–231.*
9 Bohm K. H. (1971) *Zbl. Bakt. I. Orig. 218. 330–334.*

AZIDE DEXTROSE BROTH (ROTHE)

Code: CM868

For the detection of enterococci in water.

Formula	gm/litre
Peptone	20.0
Glucose	5.0
Sodium chloride	5.0
Di-potassium hydrogen phosphate	2.7
Potassium dihydrogen phosphate	2.7
Sodium azide	0.2
Final pH 6.8 ± 0.2	

Directions
Add 35.6g to one litre of distilled water for single strength broth or 71.2g for double strength broth. Heat gently to dissolve. Dispense into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
Azide Dextrose Broth (Rothe) is used for the detection of enterococci in water and sewage[1].

The presence of enterococci serves as an indicator of faecal contamination. Enterococci are better indicators than *E. coli* of sewage pollution in chlorinated waters because they have a greater resistance to chlorine.

Mallmann and Seligmann[2] recommended Azide Dextrose Broth for the quantitative determination of enterococci in water, sewage, foods and other materials suspected of contamination with sewage.

A blend of peptone and glucose render Azide Dextrose Broth highly nutritious, and sodium chloride maintains osmotic equilibrium. The use of sodium azide as an inhibitor of Gram-negative organisms has been reported by several workers[2,3,4], and the concentration selected provides optimum protection for the enterococci while largely suppressing the Gram-negative flora. The phosphate buffer system controls pH.

Technique
Inoculate 10ml of medium with 1ml of the test sample. Inoculate a further three tubes with 0.1ml, 0.01ml and 0.001ml sample respectively. For samples of 10ml or more, use double strength broth. Incubate all tubes at 35°C and examine for turbidity after 24 and 48 hours. For a more detailed description please consult 'Standard Methods for the Examination of Water and Wastewater'.

The presence of enterococci in the sample is indicated by turbidity in the broth. Positive cultures should be inoculated into Ethyl Violet Azide Broth (Litsky) CM869 to confirm the presence of enterococci.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control
Enterococcus faecalis ATCC® 29212

Negative control
Escherichia coli ATCC® 25922

Precautions
This product contains less than 1% azide and has low toxicity. However, when handling the powder, wear gloves, mask and eye protection. When washing azide products down sinks, use sufficient water to prevent accumulation of azide in the plumbing.

References

1 Greenberg A. E. et al (ed). (1985) Standard Methods for the Examination of Water and Wastewater, 16th ed. APHA, Washington, D.C.
2 Mallmann W. L. and Seligmann E. B. (1950) *Am. J. Public Health 40. 286.*
3 Edwards S. J. (1933) *J. Comp. Path. Therap. 46. 211.*
4 Hartman G. (1937) *Milchw. Forsch. 18. 166.*

BACILLUS CEREUS SELECTIVE AGAR BASE

Code: CM617

A selective and diagnostic medium for the isolation and enumeration of Bacillus cereus.

Formula	gm/litre
Peptone	1.0
Mannitol	10.0
Sodium chloride	2.0
Magnesium sulphate	0.1
Disodium hydrogen phosphate	2.5
Potassium dihydrogen phosphate	0.25
Bromothymol blue	0.12
Sodium pyruvate	10.0
Agar	14.0
pH 7.2 ± 0.2	

BACILLUS CEREUS SELECTIVE SUPPLEMENT

Code: SR99

Vial contents (each vial is sufficient for 500ml of medium)

Polymyxin B	50,000 IU

Directions
Suspend 20.5g in 475ml of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of Oxoid Bacillus cereus Selective Supplement SR99 reconstituted with 2ml of sterile distilled water, then add 25ml of sterile Egg Yolk Emulsion SR47. Mix well and pour into sterile petri dishes.

Description
Bacillus Cereus Selective Agar CM617, is based on the highly specific diagnostic and selective PEMBA medium, developed by Holbrook and Anderson[1] for the isolation and enumeration of *Bacillus cereus* in foods. It meets the requirements for a medium that is sufficiently selective to be able to detect small numbers of *B. cereus* cells and spores in the presence of large numbers of other food contaminants. The medium is also sufficiently diagnostic that colonies of *B. cereus* are readily identified and confirmed by microscopic examination.

The role of *B. cereus* in food poisoning, particularly from the consumption of contaminated rice, is now well documented.[2,3,4] The organism has also been implicated in eye infections[5,6] and a wide range of other conditions including abscess formation, meningitis, septicaemia and wound infection. *B. cereus* is recognised as a significant pathogen in post-operative and post-traumatic wounds of orthopaedic patients.[7]

Amongst veterinarians, *B. cereus* is a known cause of disease, especially mastitis, in ewes and heifers.[8]

In the formulation of Bacillus cereus Selective Agar a peptone level of 0.1% and the addition of sodium pyruvate improve egg yolk precipitation and enhance sporulation. Bromothymol blue is added as a pH indicator to detect mannitol utilisation. The medium is made selective by addition of Bacillus cereus Selective Supplement SR99, which gives a final concentration of 100 IU of polymyxin B per ml of medium. Polymyxin B as a selective agent for the isolation of *B. cereus* has been previously suggested by Donovan[9] and found to be satisfactory by Mossel.[10] It is recommended that, where large numbers of moulds are expected in the inoculum, filter-sterilised cycloheximide is added to the medium at a final concentration of 40µg/ml.

The primary diagnostic features of the medium are the colonial appearance, precipitation of hydrolysed lecithin and the failure of *B. cereus* to utilise mannitol.

The typical colonies of *B. cereus* are crenated, about 5mm in diameter and have a distinctive turquoise to peacock blue colour surrounded by a good egg yolk precipitate of the same colour.

These features distinguish *B. cereus* from other *Bacillus* species except *B. thuringiensis*. Other egg yolk-reacting organisms which can grow on the medium, including *Staphylococcus aureus, Serratia marcescens* and *Proteus vulgaris* are distinguished from *B. cereus* by colony form and colour. These organisms also produce an egg yolk-clearing reaction in contrast to egg yolk precipitate produced by *B. cereus*.

Microscope examination for presence of lipid globules in the vegetative cells is recommended as a rapid and confirmatory test for *B. cereus* and replaces the need for biochemical testing. Holbrook and Anderson[1] have confirmed that only *B. cereus* of the *Bacillus* species are capable of possessing lipid globules in their vegetative cells when grown on the selective medium. One further advantage of this test is that strains of *B. cereus* that react only weakly or not at all with egg yolk can be detected and confirmed.

Technique

1 Homogenise 10g of the food sample for 30 seconds in 90ml of 0.1% Peptone Water CM9 using a Stomacher 400.[11] Dried foods should first be rehydrated by soaking 20g in 90ml of Tryptone salt solution (Tryptone L42 0.3% and sodium chloride 0.8%, pH 7.3) for 50 minutes at room temperature. Add a further 90ml of 0.1% peptone water to give a final dilution of 10^{-1}. Homogenise for 30 seconds using the Stomacher 400.
2 Further dilutions of the homogenate should be made in 0.1% peptone water.
3 Inoculate 0.1ml amounts of the 10^{-1} and higher dilutions on to the surface of the medium.
4 Incubate the plates at 35°C for 24 hours.
5 Examine for typical colonies of *B. cereus.*
6 Leave the plates for a further 24 hours at room temperature in order to detect all the *Bacillus cereus* colonies.
7 Confirm the presumptive identification of *B. cereus* by the Rapid Confirmatory Staining Procedure.
8 Report the results as the number of *B. cereus* colonies per gram weight of the food sample.

The medium may also be used for detecting *B. cereus* in milk. When necessary, decimal dilutions of the samples should be made in 0.1% peptone water. Undiluted and diluted samples are inoculated directly on to plates of agar and incubated. An incubation temperature of 30°C for 18 hours is recommended as optimal for promoting the growth of *B. cereus* relative to that of other organisms.[9]

For examining clinical specimens plates may be inoculated in the usual way.

Rapid Confirmatory Staining Procedure

This staining method was developed by Holbrook and Anderson[1] combining the spore stain of Ashby[12] and the intracellular lipid stain of Burdon.[13] For reasons of safety, Citroclear* replaces xylene in the original technique.

Procedure

1 Prepare films from the centre of a 1 day old colony or from the edge of a 2 day colony.
2 Air-dry the film and fix with minimal heating.
3 Flood the slide with aqueous 5% w/v malachite green and heat with a flaming alcohol swab until steam rises. Do not boil.
4 Leave for 2 minutes without re-heating.
5 Wash the slide with running water and blot dry.
6 Flood the slide with 0.3% w/v Sudan black in 70% ethyl alcohol. Leave for 15 minutes.
7 Wash the slide with running Citroclear* from a wash bottle for 5 seconds.
8 Blot dry using filter paper.
9 Flood the slide with aqueous 0.5% w/v safranin for 20 seconds.
10 Wash under running water.
11 Blot dry and examine under the microscope using the oil immersion lens. A blue filter may be used to accentuate the appearance of the lipid granules but this will give a blue colour cast to the red of the cytoplasm.

* Citroclear is available from:

H.D. Supplies
44 Rabans Close
Rabans Lane Industrial Estate
Aylesbury
Buckinghamshire
HP19 3RS

Characteristic appearance of *B. cereus* vegetative cells.

(i) Cells are 4–5 micron long and 1.0–1.5 micron wide with square ends and rounded corners.
(ii) The spores stain pale green to mid green, are central or paracentral in position and do not swell the sporangium.
(iii) Lipid globules are black and the vegetative cytoplasm red.

The appearance, together with the typical colony form, confirms the identification of *B. cereus.*

Storage conditions and Shelf life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

The prepared medium may be stored at 2–8°C.

Quality Control

Positive Control:
Bacillus cereus ATCC® 10876
Bacillus subtilis ATCC® 6633 (should be readily differentiated by colony form and colour)

Negative control:
Bacillus coagulans ATCC® 7050

Precautions

On this medium *B. cereus* is indistinguishable from *B. thuringiensis.*

Identify *B. cereus* by colony form, colour, egg yolk hydrolysis and confirm with cell and spore morphology.[14]

Occasional strains of *B. cereus* show weak or negative egg yolk reactions.

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BAIRD-PARKER AGAR BASE

Code: CM275

A selective and diagnostic medium for the isolation and enumeration of Staphylococcus aureus in foods.

Formula	gm/litre
Tryptone	10.0
'Lab-Lemco' powder	5.0
Yeast extract	1.0
Sodium pyruvate	10.0
Glycine	12.0
Lithium chloride	5.0
Agar	20.0
pH 6.8 ± 0.2	

Directions
Suspend 63g in one litre of distilled water and boil to dissolve the medium completely. Dispense into tubes or flasks and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add 50ml of Egg Yolk-Tellurite Emulsion SR54. Mix well before pouring.

Prepared plates may be stored at 4°C.

Description (with E-Y-T Emulsion SR54)
Baird-Parker[1] developed this medium from the tellurite-glycine formulation of Zebovitz et al.[2] and improved its reliability in isolating *Staph. aureus* from foods.

Baird-Parker added sodium pyruvate, to protect damaged cells and aid their recovery[2] and egg yolk emulsion as a diagnostic agent. It is now widely recommended by national and international bodies for the isolation of *Staph. aureus*[4].

The selective agents glycine, lithium and tellurite have been carefully balanced to suppress the growth of most bacteria present in foods, without inhibiting *Staph. aureus*.

Egg yolk emulsion makes the medium yellow and opaque. *Staph. aureus* reduces tellurite to form grey-black shiny colonies and then produces clear zones around the colonies by proteolytic action. This clear zone with typical grey-black colony is diagnostic for *Staph. aureus*. On further incubation, most strains of *Staph. aureus* form opaque haloes around the colonies. and this is probably the action of a lipase. Not all strains of *Staph. aureus* produce both reactions. Some strains of *Staph. saprophyticus* produce both clear zones and opaque haloes but experienced workers can distinguish these from *Staph. aureus* by the longer incubation time required[5].

Colonies typical of *Staph. aureus* but without an egg yolk reaction should also be tested for coagulase production[6].

Egg yolk reaction negative strains of *Staph. aureus* may occur in some foods, especially cheese.

Smith and Baird-Parker[7] found that the addition of 50µg of sulphametazine per ml of medium suppressed the growth and swarming of *Proteus* species. Small numbers of *Staphylococcus aureus* could then be recovered from specimens containing mixed *Proteus* strains.

Baird-Parker and Davenport[8] showed that the recovery of damaged staphylococci was greater on Baird-Parker medium than on other recovery media tested.

Broeke[9] and de Waart et al.[10] found Baird-Parker medium valuable in ecological studies on foods incriminated in staphyloenterotoxicosis. 97.5% of the 522 strains of *Staph. aureus* tested, isolated from human and food origins developed characteristically and quantitatively on Baird-Parker medium.

Colony characteristics of typical organisms on Baird-Parker Egg Yolk-Tellurite Medium

Organism
Staph. aureus

Growth
Good

Colony
Grey-black shiny convex 1–1.5mm diameter (18 hours) up to 3mm (48 hours) narrow white entire margin surrounded by zone of clearing 2–5mm.

Organism
Staph. epidermidis

Growth
Variable

Colony
Not shiny black and seldom produces clearing.

Organism
Staph. saprophyticus

Growth
Variable

Colony
Irregular and may produce clearing. Wide opaque zones may be produced in 24hrs.

Organism
Micrococcus species

Growth
Variable

Colony
Very small in shades of brown and black. No clearing.

Organism
Bacillus species

Growth
Variable

Colony
Dark brown matt with occasional clearing after 48hrs.

Organism
Escherichia coli.

Growth
Variable

Colony
Large brown-black.

Organism
Proteus species

Growth
Variable

Colony
Brown-black with no clearing.

Organism
Yeasts.

Growth
Variable

Colony
White, no clearing.

Technique
1 Dry the surface of agar plates for a minimal period of time prior to use.
2 With a glass spatula, spread 0.1ml aliquots of food dilutions made up in buffered peptone water on the agar surface until it is dry. Up to 0.5ml may be used on larger dishes (24 cm).
3 Incubate the inverted dishes at 35°C. Examine after 24 hours and look for typical colonies of *Staph. aureus*. Re-incubate negative cultures for a further 24 hours.

Quantitative results
Incubate the dishes for 48 hours and select those with 20–200 colonies.

Count the *Staph. aureus*-like colonies and test them for coagulase reaction.

Report *Staph. aureus* results per gram of food.

RPF SUPPLEMENT

Code: SR122

This supplement is used as an alternative to Egg Yolk-Tellurite Emulsion SR54 in Baird-Parker medium to identify coagulase-positive staphylococci.

Formula (per vial sufficient for 100ml of medium)

Bovine fibrinogen	0.375g
Rabbit plasma	2.5ml
Trypsin inhibitor	2.5mg
Potassium tellurite	2.5mg

Directions
To one vial aseptically add 10ml of sterile distilled water. Turn the vial end-over-end to dissolve. Avoid frothing the solution. Dissolution is not obtained immediately. Leave for one to two hours to dissolve completely. Aseptically add the vial contents to 90ml of sterile Baird-Parker Agar Base (Oxoid CM275) cooled to 48°C. Mix well and use immediately.

Description (with RPF Supplement SR122)

A recognised disadvantage of Baird-Parker medium with egg yolk is that the identity of the staphylococci isolated must be confirmed as *Staph. aureus* with a coagulase reaction. Also there are some strains of *Staph. aureus* which give negative egg yolk reactions and therefore all suspicious colonies should be tested.[11,12]

Attempts were made to substitute pig plasma for egg yolk in Baird-Parker medium, to obtain an in-situ coagulase test[13]. Hauschild[14] improved the reliabilty of the coagulase reaction by adding bovine fibrinogen and trypsin inhibitor to the pig plasma. Beckers et al.[15] showed that rabbit plasma used in place of pig plasma overcame the problems of false positive and false negative reactions in the medium. A rabbit plasma- fibrinogen-trypsin inhibitor-tellurite supplement for Baird-Parker medium was created by Beckers et al.[15] which gave reliable coagulase reactions for *Staph. aureus*.

Sawhney[16] observed variation in yields of *Staph. aureus* cultures when comparing RPF supplement with egg yolk supplement. He showed that it was necessary to reduce the tellurite level in the RPF supplement because it lacked the protective factor(s) in egg yolk.

Oxoid RPF supplement SR122 contains the recommended reduced level of tellurite.

The addition of RPF supplement to Baird-Parker medium gives a translucent agar plate in which the opaque zones of the coagulase reaction can clearly be seen.

THIS IS THE DIAGNOSTIC REACTION. DO NOT ADD EGG YOLK TELLURITE EMULSION.

Colony characteristics on Baird-Parker RPF Medium
It is important to note that the reduction in tellurite level means that black colonies may not be formed. *Staph. aureus* colonies may be **white**, **grey** or **black**, surrounded by an opaque halo of fibrin precipitation i.e. the coagulase reaction. *Staph. epidermidis* will not show the coagulase reaction at 24 hours incubation but may produce zones at 40 hours.

Most *Staph. aureus* cultures will be detected at 24 hours incubation.

Technique
Surface Inoculation Method
1 Prepare the RPF Agar plates as directed.
2 Process the food sample in a stomacher or Waring blender using the recommended sample size and diluent.
3 Separate plates are inoculated with 0.1ml of the prepared samples and the subsequent decimal dilutions of them.
4 Incubate at 35°C and examine after 24 and 48 hours incubation.
5 Count all the colonies that have an opaque halo of precipitation around them. Do not limit the count to black colonies.
6 Report as number of coagulase positive staphylococci isolated per gram of food.

Pour Plate Method

1 Prepare the RPF Agar as directed and hold at 48°C.
2 Process the food sample in a stomacher or Waring blender using the recommended sample size and diluent.
3 Add 1ml of the prepared sample (initial suspension and subsequent decimal dilution) into each sterile petri dish.
4 Add aseptically 20ml of sterile RPF Agar and prepare pour plates.
5 Incubate at 35°C and examine after 24 to 48 hours.
6 Count all the colonies that have an opaque halo of precipitation around them.
7 Report as the number of coagulase positive staphylococci per gram of food.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Prepared plates of medium are best used freshly prepared[17].

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923

Negative control:
Bacillus subtilis ATCC® 6633
Staphylococcus epidermidis ATCC® 155

Precautions
Regard all suspicious colonies as *Staph. aureus* regardless of negative reactions in the medium and carry out further tests.

Colonies of some contaminating organisms growing in close proximity to the coagulase positive colonies may partially digest the coagulase halo reaction.

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BIGGY AGAR

Code: CM589

For the isolation and presumptive identification of Candida species.

Formula	gm/litre
Yeast extract	1.0
Glycine	10.0
Glucose	10.0
Sodium sulphite	3.0
Bismuth ammonium citrate	5.0
Agar	13.0
pH 6.8 ± 0.2	

Directions
Suspend 42g in 1 litre of distilled water and bring gently to the boil to dissolve the agar. Allow to cool to 50–55°C. Mix gently to disperse the flocculant precipitate and pour into sterile petri dishes.

DO NOT AUTOCLAVE THE MEDIUM.

Description
BIGGY, Bismuth Sulphite Glucose Glycine Yeast Agar, is based on the formulation developed by Nickerson[1] and may be used for the isolation and presumptive identification of *Candida* species.

In a study of sulphite reduction by yeasts, the ability of many yeasts to reduce a bismuthyl hydroxy polysulphite was noted. This was demonstrated to be most evident in *Candida* species. but strong reducing ability was confined to *Candida albicans*, *Candida krusei* and *Candida tropicalis*. Growth on an acidic or neutral medium containing bismuth sulphite produced black colonies because of the extra-cellular reduction of the bismuth sulphite, to bismuth sulphide.

The bismuth sulphite complex confers a high degree of selectivity to the medium, and most strains of bacteria are inhibited on BIGGY Agar.

Barr and Collins[2] described the addition of neomycin sulphate to the medium at 2mg per litre to improve inhibition of accompanying bacterial flora.

The medium may be used for the isolation and presumptive identification of *C. albicans* and *C. tropicalis* from sputum[2,3] and vaginal smears[4]. It is a recommended medium for the quality assessment of pharmaceutical and cosmetic products[5].

Technique
Reconstitute the medium as directed and pour into sterile petri dishes to contain approximately 20ml of medium.

Freshly prepared plates should be used. Reactions on slant cultures are unsatisfactory[1].

Incubate the plates at 28–30°C and examine daily for evidence of sulphite reduction.

Colony appearance on BIGGY Agar (48 hours)

C. albicans
Colony morphology
Smooth, circular brown-black, slight mycelial fringe; no colour diffusion into surrounding medium; no sheen.

C. tropicalis
Colony morphology
Smooth, dark brown with black centres; slight mycelial fringe; diffuse blackening of medium after 72 hours; sheen.

C. krusei
Colony morphology
Large, flat, wrinkled silvery brown-black with brown peripheries; yellow halo diffused into medium.

C. pseudotropicalis
Colony morphology
Medium size, flat, dark reddish-brown glistening; slight mycelial fringe; no diffusion.

C. parakrusei
Colony morphology
Medium size, flat, wrinkled, glistening dark reddish-brown with lighter periphery; extensive yellow mycelial fringe.

C. stellatoidea
Colony morphology
Medium size, flat, dark brown; very light mycelial fringe.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Medium should be freshly prepared just prior to use.

Quality Control
Positive control:
Candida albicans ATCC® 10231
Candida tropicalis ATCC® 750

Negative control:
Escherichia coli ATCC® 25922
Staphylococcus aureus ATCC® 25923

Precautions
Carry out further tests to confirm identity of isolated yeasts.

Do not use slants of medium because the reactions are unsatisfactory.

The flocculent precipitate present in the molten medium must be evenly suspended whilst dispensing the agar.

References

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BILE AESCULIN AGAR

Code: CM888

A differential medium for the isolation and presumptive identification of enterococci/Group D streptococci.

Formula	gm/litre
Peptone	8.0
Bile salts	20.0
Ferric citrate	0.5
Aesculin	1.0
Agar	15.0
pH 7.1 ± 0.2	

Directions
Suspend 44.5g in 1 litre of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
The major use of Bile Aesculin Agar is to differentiate between enterococci/Group D streptococci and non Group D streptococci. It may also be used for the presumptive identification of other groups of organisms.

Enterococci/Group D streptococci hydrolyse aesculin to form aesculetin and dextrose. Aesculetin combines with ferric citrate in the medium to form a dark brown or black complex which is indicative of a positive result. Bile salts will inhibit Gram-positive bacteria other than enterococci/Group D streptococci.

The value of bile tolerance together with hydrolysis of aesculin as a means of presumptively identifying enterococci/Group D streptococci is widely recognised[1–5].

The use of these parameters forms the basis of Bile Aesculin Agar and was described by Swan[6] who concluded that the use of this medium is a valid alternative to Lancefield grouping for the recognition of enterococci/Group D streptococci.

Facklam[7] further confirmed its usefulness in differentiating enterococci/Group D streptococci from non Group D streptococci while other workers have used the medium for presumptive identification of the Klebsiella-Enterobacter-Serratia group amongst the Enterobacteriaceae[8–10].

Technique
Using a sterile loop inoculate the medium with 4–5 colonies and incubate at 37°C for 18–24 hours.

The result is positive for bile salt tolerance and aesculin hydrolysis if blackening of the medium occurs.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Quality Control
Positive Control:
Enterococcus faecalis ATCC® 19433
Enterobacter aerogenes ATCC® 13048

Negative Control:
Streptococcus pyogenes ATCC® 19615

References
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BISMUTH SULPHITE AGAR

Code: CM201

A modification of the original Wilson and Blair Medium for the isolation of Salmonella typhi and other salmonellae. It is particularly useful for the isolation of lactose-fermenting salmonellae.

Formula	gm/litre
Peptone	5.0
'Lab-Lemco' powder	5.0
Glucose	5.0
Disodium phosphate	4.0
Ferrous sulphate	0.3
Bismuth sulphite indicator	8.0
Brilliant green	0.016
Agar	12.7
pH 7.6 $\pm$ 0.2	

Directions (half litre volume)
Suspend 20g in 500ml of distilled water in a 1 litre flask. Heat gently with frequent agitation until the medium just begins to boil and simmer for 30 seconds to dissolve the agar. Cool to 50°–55°C, mix well to disperse suspension and pour thick plates (25ml medium per plate). Allow the medium to solidify with the dish uncovered. Larger volumes may be prepared if great care is taken and adequate head space provided.

Dry the plates before use but take care to avoid overdrying. Correctly prepared plates should have a smooth, cream-like opacity with a pale straw colour. There should be no sedimentation of the indicator.

DO NOT OVERHEAT – DO NOT AUTOCLAVE

Description
Bismuth Sulphite Agar is a modification of the original Wilson and Blair[1] selective medium for the isolation and preliminary identification of *Salmonella typhi* and other salmonellae from pathological material, sewage, water supplies, food and other products suspected of containing these pathogens.

In this medium freshly precipitated bismuth sulphite acts together with brilliant green as a selective agent by suppressing the growth of coliforms, whilst permitting the growth of salmonellae. Sulphur compounds provide a substrate for hydrogen sulphide production, whilst the metallic salts in the medium stain the colony and surrounding medium black or brown in the presence of hydrogen sulphide.

Atypical colonies may appear if the medium is heavily inoculated with organic matter. Such a situation may be prevented by suspending the sample in sterile saline and using the supernatant for inoculation.

The freshly prepared medium has a strong inhibitory action[2] and is suitable for heavily contaminated samples. Storing the poured plates at 4°C for 3 days causes the medium to change colour to green, making it less selective with small numbers of salmonellae being recovered[3]. However, for *Salmonella typhi* recovery the latter technique is not recommended[4].

Where the number of salmonellae is expected to be small, enrichment methods may be employed.

The use of this medium is advocated by several authorities[5,6,7].

Technique
Bismuth Sulphite Agar may be used in conjunction with other selective enteric agars for the isolation of salmonellae by direct plating or from enrichment media[8]. Thus the following scheme may be adopted.

Inoculate directly on Bismuth Sulphite Agar and one or more of the following:

Desoxycholate Citrate Agar CM227 or DCLS Agar CM393

XLD Agar CM469

Brilliant Green Agar CM329

MacConkey Agar No.3 CM115

At the same time inoculate an enrichment broth, such as Selenite Broth Base CM395 + Sodium Biselenite L121 or Tetrathionate Broth CM343. Subculture on to Bismuth Sulphite Agar and any other selective medium after 12–18 hours incubation. Examine the plates after 18 hours incubation and subculture suspect colonies to identification media, e.g. Kligler Iron Agar CM33.

All negative plates should be incubated for 48 hours.

Salmonella typhi
Appearance
Black 'rabbit-eye' colonies with a black zone and metallic sheen surrounding the colony after 18 hours. Uniformly black after 48 hours incubation.

Other Salmonella species
Appearance
Variable colony appearance after 18 hours, they may be black, green or clear and mucoid. Uniformly black colonies are seen after 48 hours, often with widespread staining of the medium and a pronounced metallic sheen.

Other organisms, e.g. coliform bacteria, *Serratia, Proteus* species
Appearance
Usually inhibited but occasional strains give dull green or brown colonies with no metallic sheen or staining of the surrounding medium.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label. Note the following comments:

Due to its contents of reactive and hygroscopic substances, dehydrated Bismuth Sulphite Agar quickly deteriorates when exposed to the atmosphere. This is usually indicated by aggregation into a solid non-friable mass, and by the development of a brown coloration. Medium reconstituted from such material is brown, does not become green on storage, and is characterised by loss of differential and selective properties. For this reason the powder should be stored in a cool, dry place and after use the container should be properly closed.

Prepared medium
It is recommended that the medium should be used on the day of preparation.

Quality Control
Salmonella typhi should be used only in a Class II laboratory, not for routine testing or in food laboratories.

Positive Control:
Salmonella enteritidis ATCC® 13076
S. typhi-murium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922
Citrobacter freundii ATCC® 8090

Precautions
Prepared plates of medium should not be stored for longer than two days at 2–8°C; after which time the dye oxidises to give a green medium that can be inhibitory to some salmonellae.

Shigella species are usually completely inhibited.

Salmonella sendai, *S. cholera-suis*, *S. berta*, *S. gallinarum* and *S. abortus-equi* are markedly inhibited[9,8].

It is important that the spreading technique yields well separated colonies. The typical colonial characteristics will not develop if the growth is too heavy or confluent; *S. typhi* colonies will appear light green in these circumstances. Therefore, when in doubt, almost any growth on the medium should be subject to further tests.

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BLOOD AGAR BASE

Code: CM55

A non-selective general purpose medium which may be enriched with blood or serum.

Formula	gm/litre
'Lab-Lemco' powder	10.0
Peptone Neutralised	10.0
Sodium chloride	5.0
Agar	15.0
pH 7.3 ± 0.2	

Directions
Suspend 40g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

For blood agar, cool the Base to 50°C and add 7% of Defibrinated Horse Blood SR50. Mix with gentle rotation and pour into petri dishes or other containers.

Description
Oxoid Blood Agar Base is a non-selective general purpose medium widely employed for the growth of pathogenic and non-pathogenic bacteria:

(i) Without additions, the medium may be employed as a nutrient agar (a richer medium than Nutrient Agar CM3), or as a medium for the short-term maintenance of stock cultures.

(ii) With added serum or other enrichments, the medium becomes suitable for the cultivation of many fastidious organisms. Serum and other thermolabile enrichments should be added to the sterilised medium cooled to 45–50°C.

(iii) With added blood, the medium is not only enriched, but becomes suitable for the determination of the typical haemolytic reactions which are important diagnostic criteria for streptococci, staphylococci, and other organisms. For blood agar, 7% of sterile blood should be added to the sterilised medium cooled to 45–50°C.

Blood Agar Base was used during investigations on irradiated *Escherichia coli* and other bacteria[1,2]. It was the most suitable medium for investigating the phages of *Clostridium perfringens*[3] and as the basis of a selective medium for *Cl. perfringens*[4]. It was used with added phenolphthalein phosphate for the detection of phosphatase-producing staphylococci[5] and with added salt and agar for the assessment of surface contamination on equipment and pig carcasses[6]. It was used for determining the salinity range of growth of marine flavobacteria[7].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium at 2–8°C.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923
Streptococcus pyogenes ATCC® 19615
Streptococcus pneumoniae ATCC® 6303

Negative control:
Uninoculated plate.

Precautions
The haemolytic reactions of organisms inoculated on to this medium will be affected by the animal blood used e.g. horse or sheep and the incubation conditions e.g. aerobic, capnoeic or anaerobic[8].

When horse blood is added to the medium *Haemophilus haemolyticus* colonies will produce beta-haemolysis and mimic *Streptococcus pyogenes*[8].

References

1 Alper T. and Gillies N. E. (1960) *J. Gen. Microbiol. 22 113–128.*
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3 Williams Smith H. (1959) *J. Gen. Microbiol. 21 622–630.*
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BLOOD AGAR BASE NO.2

Code: CM271

An improved Blood Agar Base possessing enhanced nutritional properties suitable for the cultivation of fastidious pathogens and other micro-organisms.

Formula	gm/litre
Proteose peptone	15.0
Liver digest	2.5
Yeast extract	5.0
Sodium chloride	5.0
Agar	12.0
pH 7.4 ± 0.2	

Directions
Suspend 40g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 45–50°C and add 7% sterile blood.

Mix with gentle rotation and pour into petri dishes or other containers.

Description
Oxoid Blood Agar Base No.2 was developed to meet the demand for an especially nutritious blood agar base which would permit the maximum recovery of delicate organisms without interfering with their haemolytic reactions. In comparison with fresh digest agar, Blood Agar Base No.2 may be shown to have equal or superior growth promoting properties and chromogenic bacteria grown on the Oxoid medium show enhanced pigment formation. Comparison with many other blood agars has shown that with Oxoid Blood Agar Base No.2 growth of many bacteria – especially the fastidious streptococci and pneumococci – is considerably improved, as shown by luxuriant and early colonial development.

Oxoid Blood Agar Base No.2 is specified by the American Food and Drug Administration for the preparation of sheep blood agar.[1]

Phillips[2], described an improved medium for sporulation of *Clostridium perfringens* based on Blood Agar Base No.2 to which are added lysed horse blood, bile, sodium bicarbonate and quinoline.

The medium induced significant sporulation in all of 100 strains of *Clostridium perfringens* isolated from human faeces.

Brucella:
To prepare a selective medium add Brucella Selective Supplement SR83 to 500ml of sterile, molten Blood Agar Base No.2 containing 5–10% v/v inactivated horse serum and 1% w/v dextrose.[2,3]

Campylobacter:
To prepare a selective medium add Campylobacter Supplement (Skirrow) 5 SR69 or Campylobacter Supplement (Butzler) 6 SR85 or Campylobacter Supplement (Blaser-Wang)[7] to 500ml of sterile, molten Blood Agar Base No.2 containing Campylobacter Growth Supplement SR848 and 5–7% v/v horse blood.

Haemophilus:
For the primary isolation of *Haemophilus* species from specimens containing a mixed flora, use Blood Agar Base No.2 with added Defibrinated Horse Blood SR50. Even better results may be obtained using the horse blood agar plates with half of each spread with 2 drops of 10% saponin.[9] Where haemolytic reactions are not important, for example when dealing with pure cultures, the Base may be used to prepare chocolate agar. Add 10% of Defibrinated Horse Blood code SR50 to the Base at 80°C and maintain at this temperature for 5 to 10 minutes, agitating frequently. Cool to 50°C, mix well and pour plates.

Roberts, Higgs and Cole used Blood Agar Base No.2 as the basis of a medium which is selective for *Haemophilus* spp. in primary culture of clinical specimens. The medium distinguishes *Haemophilus influenzae* and *Haemophilus parainfluenzae* by differences in colony colour.[10]

A selective chocolate blood agar for the culture of *Haemophilus influenzae* from respiratory secretions of cystic fibrosis patients has been described.[11] The medium is based on Blood Agar Base No. 2 to which 7% v/v horse blood and 8mg/litre of cefsulodin is added. Growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* on this medium is inhibited.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium at 2–8°C.

Quality Control
Blood Agar
Positive control:
Staphylococcus aureus ATCC® 25923
Streptococcus pyogenes ATCC® 19615
Haemophilus influenzae ATCC® 35056

Negative control:
Uninoculated plate.

Brucella Agar
Positive control:
Brucella abortus ATCC®4315

Negative control:
Escherichia coli ATCC® 25922

Campylobacter Agar
Positive control:
Campylobacter jejuni ATCC® 29428

Negative control:
Escherichia coli ATCC® 25922

Precautions
Brucella cultures are highly infective and must be handled under properly protected conditions. Incubate in 5–10% carbon dioxide atmosphere for 24–48 hours.

References

1 F.D.A. *Bacteriological Analytical Manual* (1992) 7th Edition F.D.A. Washington D.C.
2 Phillips K.D. (1986) *Lett. Appl. Microbiol 3 77–79.*
3 Farrell I.D. and Robinson L. (1972) *J. Appl. Bact. 35. 625–630.*
4 Hunter D. and Kearns M. (1977) *Brit. Vet. J. 133. 486–489.*
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8 George H.A., Hoffman P.S. and Krieg N.R. (1978) *J. Clin. Microbiol. 8. 36–41.*
9 Waterworth Pamela M. (1955) *Brit. J. Exp. Path. 36. 186–194.*
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BLOOD AGAR BASE (SHEEP)

Code: CM854

A Blood Agar Base that has been specifically formulated to give improved haemolytic reactions with sheep blood.

Formula	gm/litre
Tryptone	14.0
Peptone Neutralised	4.5
Yeast extract	4.5
Sodium chloride	5.0
Agar	12.5
Final pH 7.3 ± 0.2	

Directions
Suspend 40g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add 7% sterile sheep blood.

Description
The Sheep Blood Agar Base CM854 was developed to meet the demand for an especially nutritious blood agar base which would permit the maximum recovery of organisms without interfering with their haemolytic reactions when used with sheep blood. Sheep Blood Agar Base is based on the formulation of Blood Agar Base No. 2 CM271.

Blood Agar Base No. 2 when supplemented with sheep blood was occasionally found to result in a mixed haemolytic reaction (alpha and beta haemolysis) for some Group A Streptococci (*Streptococcus pyogenes).* These mixed haemolytic reactions were due to trace amounts of fermentable carbohydrates in yeast extract and the physiological differences of sheep blood when compared to horse blood[1].

Having identified the causes of the mixed haemolytic reactions, the Sheep Blood Agar Base was formulated to be compatible with sheep blood. Comparisons with other blood agar bases supplemented with sheep blood have shown that with Sheep Blood Agar Base the growth of many bacteria – especially the fastidious streptococci – is considerably improved, and the expected beta haemolytic reaction is achieved with *Streptococcus pyogenes.*

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Streptococcus pyogenes ATCC®19615
Streptococcus pneumoniae ATCC®6303

Negative control:
Uninoculated medium.

Reference

1 Spector W S (1961) *Handbook of Biological Data, P51 and 53. In W S Spector (Ed.) Handbook of Biological Data. W B Saunder Company, Philadelphia and London.*

BORDETELLA PERTUSSIS SELECTIVE MEDIA

Selective media prepared from Charcoal Agar with the addition of cephalexin.

BASE MEDIA

CHARCOAL AGAR

Code: CM119

Formula	gm/litre
'Lab-Lemco' powder	10.0
Peptone	10.0
Starch	10.0
Charcoal bacteriological	4.0
Sodium chloride	5.0
Nicotinic acid	0.001
Agar	12.0
pH 7.4 ± 0.2	

Directions
Suspend 25.5g in 500ml of distilled water. Bring to the boil to disssolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

BORDETELLA SELECTIVE SUPPLEMENT

Code: SR82
Vial contents (each vial is sufficient for 500ml of medium)

Cephalexin	20mg

Directions
Charcoal Agar
To one vial add 2ml of sterile distilled water and dissolve the contents completely. Add this solution to 500ml of sterile, molten Charcoal Agar CM119, cooled to 50°C, together with 10% v/v defibrinated horse blood SR50. Mix well before pouring into sterile petri dishes.

Transport Medium for *B. pertussis*
The vial contents may also be added to 500ml of half strength Charcoal Agar +10% v/v lysed defibrinated horse blood SR48 for use as a transport medium for *Bordetella pertussis*.

Description
Charcoal Agar CM119 with added blood may be used for the isolation of *B. pertussis*.

Bradford and Slavin[1] concluded that poor results with the cultivation of post-nasal swabs for *B. pertussis* were due to overgrowth by competing organisms rather than the absence of *B. pertussis*.

Lacey[2] was able to confirm this. He noted that even with the addition of 0.25 IU/ml of penicillin[3], the detection of *B. pertussis* was often made tedious and difficult because of overgrowth by penicillin insensitive flora.

The fact that this occurred much more often with post-nasal rather than per-nasal swabs was felt to account for the relatively small use made of per-nasal swabs in the diagnosis of whooping cough.

Lacey[2] then found that addition of M&B 938 (4,4',diamidino-diphenylamine dihydrochloride), at a level of 12µg/ml in a partially defined agar base, together with penicillin, increased the selectivity of the medium by partially inhibiting the growth of penicillin insensitive strains of *Haemophilus, Staphylococcus* and *Neisseria* and led to a higher isolation rate for *B. pertussis*.

Addition of 40µg/ml cephalexin to the medium[4] instead of penicillin and M&B 938 makes it even more selective and significantly increases the isolation rate of *Bordetella* species. Coliform organisms are inhibited and many flora are totally suppressed; some exceptions are *Pseudomonas aeruginosa* and fungi, but for suppression of fungi the addition of 50µg/ml of amphotericin B is recommended.

The use of cephalexin instead of penicillin allows a greater recovery of stressed *B. pertussis* cells thus further increasing the isolation rate.

Cephalexin has also been recommended for use in a transport medium for *B. pertussis*. Half strength Charcoal Agar together with 10% v/v horse blood and 40µg/ml cephalexin acts both to suppress growth of contaminating bacteria and as an enrichment medium to allow an increase in the numbers of viable *B. pertussis* and *B. parapertussis* organisms.

Enhanced isolation rates for *B. pertussis* may be achieved using the enrichment transport medium followed by isolation on cephalexin – charcoal agar.

Technique
The supplemented Charcoal Agar plate may be inoculated with either post or per-nasal swabs and incubated for up to 3 days at 35°C.[5]

B. pertussis grows in the 'smooth phase' on this medium and produces small, pale, drop-like glistening colonies which may be identified by microscopy and agglutination.

A 'cough-plate' technique may be used with plates filled with supplemented Charcoal Agar.

1 Distribute 30–40ml of the complete medium in a 9–10cm petri dish. The poured plate should be bright cherry red and should not be dried.
2 Inoculate the agar by holding the opened plate about 10cms in front of the patient's mouth and asking him to cough on it. Alternatively, inoculate the plate with material collected on a naso-pharyngeal or per-nasal swab.
3 Incubate the plates at 35°C in a moist chamber to prevent desiccation of the medium.

Examine the plates for *B. pertussis* after 40 hours and twice daily thereafter. Do not discard the plates as negative until they have been incubated for six days. *B. pertussis* colonies are almost transparent with a 'bisected pearl' appearance. They are surrounded by a zone of haemolysis with an indefinite periphery.

References

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3 Fleming A. (1932) *J. Path. Bact. 35. 831.*
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BRAIN HEART INFUSION

Code: CM225

A highly nutritious infusion medium recommended for the cultivation of streptococci, pneumococci, meningococci and other fastidious organisms. Suitable for blood culture work.

Formula	gm/litre
Calf brain infusion solids	12.5
Beef heart infusion solids	5.0
Proteose peptone	10.0
Glucose	2.0
Sodium chloride	5.0
Disodium phosphate	2.5
pH 7.4 ± 0.2	

Directions
Dissolve 37g in 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A versatile liquid infusion medium which is suitable for the cultivation of streptococci, pneumococci, meningococci, and other fastidious organisms. This medium is recommended for blood culture work and, with the additions described below, for the isolation and cultivation of pathogenic fungi.

Oxoid Brain Heart Infusion is essentially a buffered infusion broth giving similar results to the brain dextrose broths originally employed for the cultivation of streptococci[1], and for the cultivation of dental pathogens[2].

The addition of 0.1% of agar will serve to reduce convection currents and so create conditions of varying oxygen tension which favour the growth and primary isolation of aerobes and anaerobes[3], while even easily cultivated organisms show improved growth[4].

Brain Heart Infusion was used in a test for the pathogenicity of streptococci[5,6] and the same medium was enriched with ascitic fluid for the cultivation of gonococci[7].

Oxoid Brain Heart Infusion is especially useful as a growth and suspension medium for staphylococci which are to be tested for coagulase production; Newman[8] employed a similar medium for this purpose in an investigation of food poisoning caused by dairy products.

A satisfactory medium for blood culture can be prepared by adding 1g of agar per litre of Brain Heart Infusion. Ensure that the agar is uniformly distributed in the sterile broth before dispensing into bottles. More conveniently, add 1 Agar Tablet CM49 to each 100ml of Brain Heart Infusion and sterilise by autoclaving for 15 minutes at 121°C. Cool to 60–70°C and mix gently to ensure uniform distribution of the agar.

Tubes of Brain Heart Infusion which are not used the same day as sterilised should be placed in a boiling water bath for several minutes to remove absorbed oxygen, and cooled rapidly without shaking, just before use.

Further supplements to improve the recovery of organisms from blood can be added before sterilisation or aseptically post-sterilisation. Co-enzyme 1 (NAD), penicillinase and p-amino benzoic acid are examples.

Brain Heart Infusion supplemented with yeast extract, haemin and menadione was consistently better in producing heavy growth of five species of *Bacteroides* than three standard anaerobic broths. Furthermore, microscopy of overnight cultures showed normal morphology in Brain Heart Infusion but abnormal morphology in the three anaerobic broths[9].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store tubed or bottled medium in the dark and below 20°C.

Quality Control
Positive controls:
Streptococcus pneumoniae ATCC® 6303
Candida albicans ATCC® 10231

Negative control:
Uninoculated medium.

References

1 Rosenow E. C. (1919) *J. Dental Research l. 205–249.*
2 Haden R. L. (1923) *Arch. Internal Med. 32. 828–849.*
3 Hitchens A. P. (1921) *J. Infectious Diseases 29. 390–407.*
4 Falk C. R. et al. (1939) *J. Bact. 37. 121–131.*
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BRAIN HEART INFUSION AGAR

Code: CM375

A solid medium which contains the highly nutritious infusions recommended for the cultivation of fastidious organisms.

Formula	gm/litre
Calf brain infusion solids	12.5
Beef heart infusion solids	5.0
Proteose peptone	10.0
Sodium chloride	5.0
Glucose	2.0
Disodium phosphate	2.5
Agar	10.0
pH 7.4 ± 0.2	

Directions
Suspend 47 grams in 1 litre of distilled water. Boil to dissolve the medium completely. Distribute into tubes or flasks and sterilise by autoclaving at 121°C for 15 minutes.

Description
Brain Heart Infusion Agar may be recommended for the cultivation of streptococci, neisseria and other fastidious organisms.

Seth[1] described the use of Oxoid Brain Heart Infusion with agar for the isolation of *N. gonorrhoeae*. Oxoid Brain Heart Infusion Agar was designed to be equivalent in performance. The addition of 10% v/v horse blood plus vancomycin 3.0µg/ml, colistin methane sulphonate 7.5µg/ml, nystatin 12.5 IU/ml and trimethoprim lactate 8.0µg/ml produced a specific medium which prevented the growth of *Proteus* species without significantly affecting *N. gonorrhoeae*.

The addition of blood and antibiotics also makes Brain Heart Infusion Agar suitable for the isolation of the tissue phase of *Histoplasma capsulatum* and other pathogenic fungi, including *Coccidioides immitis*[2,3].

For the selective isolation of fungi, without blood, cyclohexamide 0.5µg/ml and chloramphenicol 0.05µg/ml of Brain Heart Infusion Agar may be added[4,5].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium at 2–8°C.

Quality Control

with blood
Positive control:
Neisseria meningitidis ATCC® 13090
Streptococcus pneumoniae ATCC® 6303

Negative control:
Uninoculated medium.

with antibiotics
Positive control:
Aspergillus niger ATCC® 9642

Negative control:
Escherichia coli ATCC® 25922

Precautions
When using this medium to isolate *H. capsulatum*, *C. immitis* or other pathogenic fungi which can produce free infective spores, extreme care must be taken to avoid dissemination of infective particles in the laboratory. The cultures should be examined only in a closed, filtered air cabinet.

References

1 Seth A. (1970) *Brit. J. Vener. Dis. 46. 201–202.*
2 Howell A. (1948) *Public Health Reports 63. 173–178.*
3 Creitz J. R. and Puckett T. F. (1954) *Amer. J. Clin. Path. 24. 1318–1323.*
4 Ajello L., Georg L. K., Kaplan W. and Kaufman L. (1960) *in Laboratory Manual for Medical Mycology (CDC) Atlanta Ga. US.DHEW. Center for Disease Control.*
5 McDonough E. S., Georg L. K., Ajello L. and Brinkman A. (1960) *Mycopath. Mycol. Appl. 13. 113–116.*

BRILLIANT GREEN AGAR

Code: CM263

A selective medium for the isolation of salmonellae, other than S. typhi.

Formula	gm/litre
Proteose peptone	10.0
Yeast extract	3.0
Lactose	10.0
Sucrose	10.0
Sodium chloride	5.0
Phenol red	0.08
Brilliant green	0.0125
Agar	12.0
pH 6.9 ± 0.2	

Directions
Suspend 50g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Brilliant Green Agar was first described as a selective isolation medium for *Salmonella* species by Kristensen et al.[1] Kauffmann[2] modified their formula to give a highly selective plating medium for the isolation and identification of salmonellae from faeces and other pathological material, and from food and dairy products. This medium was not designed for the isolation of *Salmonella typhi* or *Shigella* species and where these may be encountered, Brilliant Green Agar should be used in parallel with other selective plating media such as Desoxycholate Citrate Agar (Hynes) CM227, Hektoen Enteric Agar CM419, XLD Agar CM469. Bismuth Sulphite Agar (Modified) CM201 is specifically recommended for *Salmonella typhi*.

The use of enrichment/selective broths prior to subculture on Brilliant Green Agar will improve the probability of isolating salmonellae. Tetrathionate Broth Base CM29, Tetrathionate Broth USA CM671, Selenite Broth Base CM395 and Muller-Kauffmann Tetrathionate Broth Base CM343 may be used in conjunction with Brilliant Green Agar.

Brilliant Green Agar corresponds to the medium recommended by the APHA[3,4] and the AOAC[5].

The addition of sulphonamides to Brilliant Green Agar helps improve the isolation of salmonellae[6]. To one litre of Brilliant Green Agar add 1.0g of sulphapyridine or 0.8g sulphadiazine and sterilise in the normal way.

Technique

Examination of faeces, or similar material, for salmonellae:
1 Heavily inoculate a Brilliant Green Agar plate. At the same time, inoculate other plating media and tubes of Selenite Broth and Tetrathionate Broth.
2 Incubate the Brilliant Green Agar plate for 18–24 hours at 35°C.
3 Examine the plates and identify suspect colonies using differential tests for serological methods.
4 If no non-lactose fermenters are observed on the primary plate cultures, inoculate Brilliant Green Agar and other media with the enrichment cultures – then proceed as in paragraph 3.

Examination of Foods
1 Pre-enrich four 25g aliquots of food in 75ml of Buffered Peptone Water CM509 and incubate at 35°C for 4–6 hours.
2 Add to each sample 75ml of double-strength Selenite Cystine Broth CM699 and incubate at 43°C for 24 hours.
3 Subculture to plates of Brilliant Green Agar and Bismuth Sulphite Agar (Modified) CM201.
4 Incubate the plates at 35°C and examine the Brilliant Green Agar after 24 hours and the Bismuth Sulphite Agar after 48 hours.
5 Look for colonies with salmonella characteristics and confirm their identity with biochemical and serological tests.

Examination of food for salmonellae (enumeration)[4]
This is carried out by adding equal volumes of decimal dilutions of the homogenised sample to tubes of double strength Selenite Broth. After incubation, a loopful from each tube is plated on Bismuth Sulphite Agar and Brilliant Green Agar. Colonies with salmonellae characteristics are identified and the most probable number of salmonellae per gram of sample is calculated from the three highest sample dilutions which yield salmonellae on subculture.

Examination of dairy products for salmonellae[3]
Milk and liquid milk products, dried milk, cheese, eggs and egg products – Brilliant Green Agar is employed, with and without an enrichment phase, in conjunction with other selective media for enteric bacteria.

Colonial Characteristics
Non-lactose/sucrose-fermenting organisms
Red-pink-white opaque coloured colonies surrounded by brilliant red zones in the agar – most probably salmonella (but not *S. typhi*).

Proteus and Pseudomonas species
These may grow as small red colonies.

Lactose/sucrose-fermenting organisms (normally inhibited)
Yellow to greenish-yellow coloured colonies surrounded by intense yellow-green zones in the agar – *E. coli* or *Klebsiella/Enterobacter* group.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium at 2–8°C.

Quality Control
Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922
Proteus vulgaris ATCC® 13315

Precautions
Lactose-fermenting salmonella *(S. arizona)* may be present in foods[7].

Salmonella typhi and *Shigella* species may not grow on this medium, use the cited alternative media.

Proteus, Citrobacter and *Pseudomonas* species may mimic enteric pathogens by producing small red colonies.

References

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BRILLIANT GREEN AGAR (MODIFIED)

Code: CM329

A selective and diagnostic agar for salmonellae (other than S. typhi) from food and feeds.

Formula	gm/litre
'Lab-Lemco' powder	5.0
Peptone	10.0
Yeast extract	3.0
Disodium hydrogen phosphate	1.0
Sodium dihydrogen phosphate	0.6
Lactose	10.0
Sucrose	10.0
Phenol red	0.09
Brilliant green	0.0047
Agar	12.0
pH 6.9 ± 0.2	

Directions
Suspend 52 grams in 1 litre of distilled water. Heat gently with occasional agitation and bring just to the boil to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 50°C, mix well and pour plates.

SULPHAMANDELATE SUPPLEMENT

Code: SR87

Vial contents (each vial is sufficient for 500ml of medium)

Sodium sulphacetamide	500mg
Sodium mandelate	125mg

Directions
To one vial add 5ml of sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Add the solution to 500ml of sterile Oxoid Brilliant Green Agar (Modified) CM329 cooled to 50–55°C. Mix gently and pour into sterile petri dishes.

Description
Brilliant Green Agar (Modified) was developed from a formula supplied by the Rijks Instituut voor de Volksgezondheid (National Institute for Public Health), Utrecht[1,2].

The medium has been widely assessed in Europe and is now used in the ISO standards[3,4,5].

The advantages claimed for the medium are the greater inhibition of *Escherichia coli* and *Proteus* species than other formulations: the restriction of growth of *Pseudomonas* species, whose colonies may resemble salmonellae on Brilliant Green Agar and cause confusion or much extra work to confirm their identity: the absence of inhibitory properties towards small numbers of salmonellae[6].

SELECTIVE BRILLIANT GREEN AGAR (MODIFIED)

Watson and Walker[7] incorporated a combination of sulphacetamide (at 1.0mg/ml) and mandelic acid (at 0.25mg/ml) into Oxoid Brilliant Green Agar (Modified) to obtain maximum recovery of salmonellae from Muller-Kauffmann Tetrathionate Broth whilst giving maximum suppression of contaminating organisms.

Oxoid Salmonella Sulpha-Mandelate Supplement, SR87 used for the isolation and enumeration of salmonellae from sewage and sewage sludge, is based on the formulation of Watson and Walker[1,7]. These authors showed that the use of Brilliant Green Agar (Modified) CM329 incorporating a combination of sulphacetamide (1.0mg/ml) and mandelic acid (0.25 mg/ml) incubated at 43°C resulted in maximum recovery of salmonellae from Muller-Kauffmann Tetrathionate Broth.

The method described[7] has been shown to be a quick and reliable technique for the isolation of sub-lethally damaged salmonellae from treated sewage and sewage sludge.

Use of antibiotic supplemented Brilliant Green Agar is made necessary because the pre-enrichment of the sewage in phosphate buffered peptone (PBP) water will encourage not only the growth of stressed salmonellae but many competing organisms.

The inhibitory properties of Muller-Kauffmann Tetrathionate Broth are not sufficient by themselves to suppress the growth of the latter. The advantage claimed for Selective Brilliant Green Agar is its greater inhibition of contaminating organisms and a lower incidence of false positives.

This advantage was confirmed by Fricker and his co-workers when using Brilliant Green Agar (Modified) CM329 containing sodium sulphacetamide and sodium mandelate for plating enrichment cultures in Rappaport Broth, from sewage and sewage polluted water[8,11], seagull faeces[9] and chicken[10,12].

Vassilliadis et al.[13] added 2.5g of sodium desoxycholate L57 to one litre of Brilliant Green Agar (Modified) to prevent swarming by *Proteus hauseri*, during examination of sewage effluents. They found desoxycholate to be superior to sulphonamides in suppressing swarming without affecting the growth of a wide range of salmonellae serotypes.

Colonial Characteristics
Salmonellae – red colonies surrounded by bright red medium.

Lactose/Sucrose fermenters – inhibited to a certain extent, but producing yellow green colonies when growth is evident.

Proteus – almost completely inhibited, those colonies that grow produce red colonies without swarming.

Pseudomonas – inhibited growth of small, crenated red colonies.

Technique
Technique for food and feeds
An outline of the method used by Edel and Kampelmacher[2] in their trials is as follows:

1 One part of the food sample was added to 20 parts of Muller-Kauffmann Tetrathionate Medium CM343.
2 After agitation, the flask of broth was placed into a 45°C waterbath for 15 minutes only.
3 The flask was then transferred to a 43°C incubator.
4 The broth was subcultured to Brilliant Green Agar (Modified) after 18 and 48 hours.

   A single loopful of broth was used to streak inoculate either two 9cm diameter plates (without recharging the loop between plates) or one 14cm diameter plate.
5 The plates were incubated at 35°C for 18–24 hours.
6 Red colonies, resembling salmonellae, were picked off the plates and subcultured to Lysine Decarboxylase Broth CM308 and Triple Sugar Iron Agar CM277. These media were incubated at 35°C for 18–24 hours.

If the reactions on these media were positive for salmonellae then slide agglutination tests were carried out on the surface growth of the Triple Sugar Iron Agar.

Technique for sewage[7]
1 Take a representative sample of sewage or sludge for examination.
2 Homogenise a suitable volume in a macerator or stomacher.
3 Inoculate five 10ml samples into 35ml of Buffered Peptone Water CM509, five 1ml samples and five 0.1ml samples into 10ml of Buffered Peptone Water. Incubate at 35°C overnight.
4 Transfer 10ml portions into 35ml of Muller-Kauffmann Tetrathionate Broth and incubate at 43°C.
5 Subculture the broths on to Brilliant Green Agar (Modified) containing Sulphamandelate Selective Supplement SR87 after 24 and 48 hours incubation.
6 Incubate the Brilliant Green Agar plates overnight at 43°C.
7 Identify suspicious (red) colonies using further diagnostic tests.

The Sulphamandelate Selective Supplement SR87 inhibits competing organisms which multiply during the resuscitation and recovery stages in Buffered Peptone Water.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium at 2–8°C.

Quality Control
Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922
Proteus vulgaris ATCC® 13315

Precautions
Lactose-fermenting salmonellae may be present in foods.

Salmonella typhi and *Shigella* species may not grow on this medium.

Proteus, *Citrobacter* and *Pseudomonas* species may mimic enteric pathogens by producing small red colonies.

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BRILLIANT GREEN BILE (2%) BROTH

Code: CM31

This medium is used to detect or confirm the presence of members of the coli-aerogenes group; the brilliant green content suppresses anaerobic lactose fermenters, such as Clostridium perfringens, and the medium is recommended for the 44°C confirmatory test for Escherichia coli.

Formula	gm/litre
Peptone	10.0
Lactose	10.0
Ox bile (purified)	20.0
Brilliant green	0.0133
pH 7.4 ± 0.2	

Directions
Dissolve 40g in 1 litre of distilled water. Mix well, distribute into containers fitted with Durham's tubes and sterilise by autoclaving at 121°C for 15 minutes.

An alternative procedure is to heat the dissolved broth at 100°C for 30 minutes, a recommended procedure when preparing double-strength broth[1].

Description
This medium was formulated by Durham and Schoenlein[2] to select organisms of the coli-aerogenes group. The bile and brilliant green components inhibit the Gram-positive organisms, whilst the coli-aerogenes group are recognised by the rapid formation of gas during lactose fermentation[3].

It is important that the inhibitory agents in the medium are balanced with the nutrient and mineral components, so that *Clostridia* and *Bacillus* spores will not give false positive reactions in the medium i.e. gas formation.

Brilliant Green Bile Broth is used in water, dairy and food analysis[4,5,6,7,8].

MUG Reagent BR71 – The addition of 4-methylumbelliferyl-β-D-glucuronide (MUG) BR71 to this medium will enhance the detection of *Escherichia coli.* See MUG Reagent BR71 under Biological Reagents for further details.

Technique
To indicate the presence of *Escherichia coli*, Brilliant Green Bile Broth is incubated at 44 ± 1°C for 48 hours. Turbidity in the broth and gas production in the inverted tube are positive signs. An indole production test at 44°C is also carried out in Tryptone Water CM87 or Peptone Water CM9 to confirm the identity of *E. coli*.

In water plant control tests where <1ml to 10ml volumes of water are used, it is important not to overdilute the medium. Thus 1ml or less volumes of water can be added to 10ml of Brilliant Green Bile Broth. For 10ml volumes of water, double-strength Brilliant Green Bile Broth should be used in equal volumes.

When incubated at 35°C for 48 hours, gas formation presumptively indicates coli-aerogenes organisms.

Food macerates are decimally diluted and added to the broth in the proportion 1:10. Double strength broth can be used for large volume samples.

Incubation is carried out at 44°C for 48 hours to detect *E. coli*. At 32°C for 25–48 hours to detect coli-aerogenes organisms[1,10] or at 4°C for 10 days to detect psychrotrophic coliform organisms.

The medium becomes turbid and yellowish-green in colour when bacteria are growing and when accompanied by copious gas formation, there is presumptive evidence of coli-aerogenes organisms. Confirmatory tests should be carried out.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared tubes of broth at 2–8°C.

Quality Control
Positive control:
Escherichia coli ATCC® 25922
Enterobacter aerogenes ATCC® 13048

Negative control:
Staphylococcus aureus ATCC® 25923
Bacillus cereus ATCC® 10876

Precautions
Do not autoclave double-strength broth.

Gram-positive sporing organisms may produce gas if the bile/brilliant green inhibition is attenuated by food material.

References

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BRUCELLA SELECTIVE MEDIA

For the selective isolation of Brucella species from milk.

BASE MEDIA

BLOOD AGAR BASE NO. 2

Code: CM271

Formula	gm/litre
Proteose peptone	15.0
Liver digest	2.5
Yeast extract	5.0
Sodium chloride	5.0
Agar	12.0
pH 7.4 ± 0.2	

Directions
Suspend 20g in 500ml of distilled water, bring to the boil to dissolve completely. Mix and sterilise by autoclaving at 121°C for 15 minutes.

Or

COLUMBIA AGAR BASE

Code: CM331

Formula	gm/litre
Special peptone	23.0
Starch	1.0
Sodium chloride	5.0
Agar	10.0
pH 7.3 ± 0.2	

Directions
Suspend 19.5g in 500ml of distilled water. Boil to dissolve the medium completely. Sterilise by autoclaving at 121°C for 15 minutes.

Or

BRUCELLA MEDIUM BASE

Code: CM169

Formula	gm/litre
Peptone	10.0
'Lab-Lemco' powder	5.0
Glucose	10.0
Sodium chloride	5.0
Agar	15.0
pH 7.5 ± 0.2	

Directions
Suspend 22.5g in 500ml of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

BRUCELLA SELECTIVE SUPPLEMENT

Code: SR83

Vial contents (each vial is sufficient for 500ml of medium)

Polymyxin B	2500 IU
Bacitracin	12500 IU
Cycloheximide	50mg
Nalidixic acid	2.5mg
Nystatin	50000 IU
Vancomycin	10mg

Directions
To one vial add 10ml of a 50:50 mixture of methanol and sterile distilled water to form a suspension. Incubate for 10–15 minutes at 35°C. Mix thoroughly and add to 500ml of sterile medium.

Use Blood Agar Base No. 2 CM271 (or Columbia Agar Base CM331) plus 5–10% v/v inactivated horse serum and 1% w/v sterile dextrose cooled to 50–55°C.

Description
The slow growth of *Brucella* species, combined with their requirement for highly nutritious media means that selective agents must be incorporated to prevent overgrowth of contaminant organisms from milk or veterinary tissues.

Jones and Brinley Morgan[1] showed that media containing bacteriostatic dyes are inhibitory to strains of *Brucella abortus* biotype 2 and other fastidious strains.

They also showed that antibiotics used in place of dyes enabled growth of all biotypes of *Brucella* species to appear on selective media. However, Leech et al.[2] showed that the serum-glucose-antibiotic formulation of Jones and Brinley Morgan was not sufficiently selective and was less efficient than guinea-pig inoculation.

Barrow and Peel[3] modified a selective medium devised by Mair[4]. This contained both antibiotics and gentian violet. Failure of some strains of *Brucella abortus* to grow confirmed their sensitivity to very low concentrations of the dye recognised by Mair.

Farrell[7] developed a highly selective antibiotic containing nutrient medium which incorporated bacitracin 25 IU/ml, vancomycin 20µg/ml, polymixin 5 IU/ml, nalidixic acid 5µg/ml, cycloheximide 100µg/ml and nystatin 100 IU/ml, in a serum-glucose agar base.

In comparative trials[5] the medium was shown to give a rate of isolation equivalent to that achieved by guinea-pig inoculation. It also supported the growth of *Brucella abortus* biotype 2 strains.

Oxoid Brucella Selective Supplement SR83 is based on the superior formulation of Farrell. Its greater efficiency at suppressing bacterial contamination than either serum glucose agar or Barrow and Peels' Medium was shown in a further trial[6].

Technique

1 For direct culture of *Brucella* species from milk transfer the samples to sterile tubes and hold overnight at 40°C.
2 Withdraw an aliquot of gravity cream with a spiral wire[4] and spread over a plate of supplemented agar with a bent sterile glass rod.
3 Incubate the plates at 35°C in an atmosphere containing 10–20% (v/v) carbon dioxide and examine every two days for ten days.
4 *Brucella* colonies appear as 1–2mm diameter convex colonies with round entire edges, and may be identified by slide agglutination.

References

1 Jones L. M. and Brinley Morgan W. J. (1958) *Bull. Wld Hlth Org. 19. 200. p.576.*
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6 Hunter D. and Kearns M. (1977) *Br. Vet. J. 133. 48&489.*
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BRUCELLA MEDIUM BASE

Code: CM169

For the preparation of serum-dextrose-antibiotic medium for the cultivation and isolation of Brucella using Brucella Selective Supplement SR83. Without antibiotics, it may be used in conjunction with the Cruickshank dyestrip method for differentiation between strains.

Formula	gm/litre
Peptone	10.0
'Lab-Lemco' powder	5.0
Glucose	10.0
Sodium chloride	5.0
Agar	15.0
pH 7.5 ± 0.2	

Directions

Suspend 45g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add 5% of inactivated Horse Serum (i.e. serum held at 56°C for 30 minutes). Mix well before pouring.

BRUCELLA SELECTIVE SUPPLEMENT

Code: SR83

Vial contents (each vial is sufficient for 500ml of medium)

Polymyxin B	2,5000 IU
Bacitracin	12,500 IU
Cycloheximide	50mg
Nalidixic acid	2.5mg
Nystatin	50,000 IU
Vancomycin	10mg

Directions

Add 10ml of a 50:50 mixture of methanol and sterile distilled water to form a suspension. Incubate for 10–15 minutes at 35°C. Mix thoroughly and immediately add the vial contents to 500ml of sterile Oxoid Brucella Medium Base CM169, cooled to 50°C together with 5–10% v/v sterile inactivated horse serum SR35 and 1.5% w/v of a filter-sterilised 10% solution of dextrose. Mix well and pour into sterile petri dishes.

Description

Oxoid Brucella Medium Base may be used to prepare the serum-dextrose-antibiotic medium described by Jones and Brinley Morgan[1] for the cultivation and isolation of *Brucella*, including fastidious types. Brucella medium with antibiotics has advantages over the media described by Kuzdas and Morse[2] and Renoux[3] in that it will support growth of fastidious types and it is more effective as a selective medium. During investigations, Jones and Brinley Morgan showed that serum-glucose agar with antibiotics gave excellent growth of all *Brucella* strains and permitted better growth of *Brucella abortus* biotype 2 – a strain which had been difficult or impossible to cultivate.

Technique

The addition of dyes (i.e. malachite green and gentian violet) as selective agents, is not recommended, as it may result in poor growth of many *Brucella* strains. Where a non-selective medium is required, the Oxoid base may be employed with the addition of serum only (i.e. without antibiotics), and for subsequent differentiation between strains of *Brucella* this medium is recommended for use in conjunction with the Cruickshank dyestrip method[4]:

1 Impregnate filter paper strips with 1:200 Basic Fuchsin or 1:600 Thionin. Dry.
2 Place the strips parallel on the surface of the serum-dextrose agar and then cover with a thin layer of the same medium. Then allow the medium to solidify.
3 Make stroke inoculations of the *Brucella* strains to be tested, at right angles to the strips.

4 Incubate in 10% carbon dioxide for 2–3 days at 35°C.

5 Examine. Resistant strains grow right across the strip, but sensitive strains show inhibition of growth up to 10mm from the strip. Typical growth patterns are then as follows:

	Basic Fuchsin 1:200	Thionin 1:600
Brucella abortus	growth	no growth
Brucella melitensis	growth	growth
Brucella suis	no growth	growth

However, there are exceptions to the above and it is therefore advisable to base identification on many characteristics[5].

The slow growth of *Brucella* species, combined with their requirement for highly nutritious media means that selective agents must be incorporated to prevent overgrowth of contaminant organisms from milk or veterinary tissues.

Media containing bacteriostatic dyes are inhibitory to strains of *Brucella abortus* biotype 2 and other fastidious strains. Antibiotics used in place of dyes enabled growth of all biotypes *Brucella* species to appear on selective media[1]. However, Leech et al.[6] showed that the serum-glucose-antibiotic formulation[1] was not sufficiently selective and was less efficient than guinea-pig inoculation.

Barrow and Peel[7] modified a selective medium devised by Mair[8]. This contained both antibiotics and gentian violet. Failure of some strains of *Brucella abortus* to grow confirmed their sensitivity to very low concentrations of the dye recognised by Mair.

Farrell[9] developed a highly selective antibiotic-containing nutrient medium which incorporated bacitracin 25iu/ml, vancomycin 20µg/ml, cycloheximide 100µg/ml and nystatin 100iu/ml, in a serum-glucose agar base.

In comparative trials[10] the medium was shown to give a rate of isolation equivalent to that achieved by guinea-pig inoculation. It also supported the growth of *Brucella abortus* biotype 2 strains.

Oxoid Brucella Selective Supplement SR83 is based on the superior formulation of Farrell. Its greater efficiency at suppressing bacterial contamination than either serum glucose agar or Barrow and Peel's Medium was shown in a further trial[11].

1 For direct culture of *Brucella* species from milk transfer the samples to sterile tubes and hold overnight at 40°C.

2 Withdraw an aliquot of gravity cream with a spiral wire and spread over a plate of supplemented agar with a bent sterile glass rod.

3 Incubate the plates at 35°C in an atmosphere containing 10–20% (v/v) carbon dioxide and examine every two days for ten days.

4 *Brucella* colonies appear as 1–2mm diameter convex colonies with round entire edges, and may be identified by slide agglutination.

References

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BUFFERED PEPTONE WATER

Code: CM509

A pre-enrichment medium to be used prior to selective enrichment for the isolation of Salmonella species from foods.

Formula	gm/litre
Peptone	10.0
Sodium chloride	5.0
Disodium phosphate	3.5
Potassium dihydrogen phosphate	1.5
pH 7.2 ± 0.2	

Directions

Add 20g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes. It is extremely important that the distilled water used is of a high quality with a low mineral content/conductivity.

Description

Oxoid Buffered Peptone Water CM509 may be used as a pre-enrichment medium, prior to selective enrichment in the isolation of salmonellae from foods. It also provides conditions for resuscitation of cells that have been injured by processes of food preservation.

It was noted by Edel and Kampelmacher[1] that sublethal injury to salmonellae may occur in many food processes. In a survey involving isolation of salmonellae from meat that had been artificially contaminated with sublethally injured organisms, pre-enrichment in buffered peptone water at 37°C for 18 hours before selection in Brilliant Green-Tetrathionate-Bile Broth showed superior results compared with a direct selection method.

Pietzsch[2] found that isolation of salmonellae was much improved by pre-enrichment of egg samples in buffered peptone water at 37°C for 18 hours followed by incubation of 10ml of this sample in 100ml Selenite Cystine Broth CM699 or Muller-Kauffmann Tetrathionate Broth CM343 at 43°C for 48 hours.

Sadovski[3] reported that in experiments involving isolation of salmonellae from frozen vegetables the rapid drop in pH when using lactose broth[4] as a pre-enrichment medium was detrimental to the recovery

of salmonellae. This was due to the enhanced sensitivity to low pH of freeze-injured salmonellae which may contaminate frozen vegetables. Pre-enrichment with buffered peptone water maintained a high pH over a period of 24 hours incubation. Vegetable tissue has a low buffering capacity and the medium overcame this problem.

A shortened enrichment time of 6 hours was investigated[6] but in circumstances where heavily contaminated materials were examined, the addition of 0.1g of malachite green per litre of Buffered Peptone Water was advised.

The addition is important where small numbers of salmonellae may have their generation time increased because of competitive growth and may not reach the minimum number for successful isolation.

For cocoa products the inclusion of casein in the pre-enrichment medium is necessary to inhibit bactericidal substances present in cocoa[7]. A comparative collaborative study confirmed the value of adding casein and malachite green to buffered peptone water when examining cocoa bean dust and chocolate for Salmonella[8].

Technique for the isolation of Salmonella[5]

1 Add 10g of sample to 50ml of Buffered Peptone Water and mix thoroughly.
2 Incubate at 35°C for 18 hours.
3 Add 10ml of incubated BPW to 100ml of Muller-Kaufmann Tetrathionate Broth CM343.
4 Incubate at 43°C.
5 Subculture to Brilliant Green Agar CM263 or Brilliant Green Agar (Modified) CM329, after 24 and 48 hours incubation.
6 Incubate the Brilliant Green Agar plates at 35°C for 18 hours.
7 Examine the plates for colonies of *Salmonella* spp.

Storage conditions and Shelf life

Store dehydrated medium below 25°C and use before the expiry date of the label.

Store prepared medium at 2–8°C.

Quality Control

Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Uninoculated medium.

Precautions

Observe the safety precautions required when cultivating salmonellae.

Liquid cultures are more infective than plates and special care should be taken if the 43°C incubation takes place in a water bath.

Do not use malachite green if *Salmonella typhi* may be present in the test material.

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CAMPYLOBACTER SELECTIVE MEDIA

The revelation in the 1970's that campylobacters are important human pathogens marked the beginning of an upsurge of interest in these organisms which has continued unabated. New species are being identified and some existing species have been assigned to new genera.

In 1977, enteritis-causing campylobacters were still in the province of a few experts but with improved media and isolation procedures, clinical laboratories can isolate thermophilic campylobacters routinely. A considerable body of literature on the genus has arisen; reference to this work and an up-date on developments has been made in a review by Penner[1]. The table below has been adapted from this review.

The major step forward in recognising the importance of campylobacters in human disease was the development of isolation media which contain antibiotics. These media suppress competing faecal flora and allowed the campylobacters to grow into easily detected colonies.

The first selective supplement was developed by Skirrow[1] and other workers followed with other antibiotic combinations.

Differential reactions and characteristics for species of the genera *Campylobacter, Arcobacter* and *Helicobacter pylori*. (Adapted from Penner[1])

Species	Catalase	Nitrate	H_2S (TSI)	Hippurate	Urease	Growth 25°C	Growth 37°C	Growth 42°C	Susceptibility Nalidixic acid	Susceptibility *Cephalothin*	*G+C content (mol%)*
Campylobacter fetus subsp. *fetus*	+	+	–	–	–	+	+	(–)	R	S	33–34
C. fetus subsp. *venerealis*	+	+	–	–	–	+	+	–	R	S	33–34
C. hyointestinalis	+	+	+	–	–	(+)	+	+	R	S	35–36
C. jejuni	+	+	–	+	–	–	+	+	S	R	30–32
C. coli	+	+	–	–	–	–	+	+	S	R	31–33
C. lari	+	+	–	–	–	–	+	+	R	R	31–33
C. upsaliensis	(–)	+	–	–	–	–	+	+	S	S	35–36
'C. cinaedi'	+	+	–	–	–	–	+	–	S	I	37–38
'C. fennelliae'	+	–	–	–	–	–	+	–	S	S	37–38
C. sputorum Biovar sputorum	–	+	(+)	–	–	–	+	+	(S)	S	31–32
Biovar bubulus	–	+	+	–	–	–	+	+	R	S	31–32
Biovar fecalis	+	+	+	–	–	–	+	+	R	S	32–33
C. mucosalis	–	+	+	–	–	–	+	+	R	S	38–39
C. concisus	–	+	+	–	–	–	+	+	R	R	38–39
Arcobacter cryaerophila	+	+	–	–	–	+	+	–	d	R	29–30
A. nitrofigilis	+	+	ND	–	+	+	+	–	S	S	28–29
Helicobacter pylori	+	d	–	–	+	–	+	+	R	S	36–37

+, Postive reaction; –, negative reaction; ND, no test results found; (+), most strains positive but a low percentage negative: (–), most strains negative but some positive or weakly positive; d, different reactions; R, resistant; S, susceptible; I, intermediate zones of inhibitions.

[a]Susceptibility to antibiotics was determined with 30µg disks.

Selective Antibiotic Supplements for the isolation of Campylobacters.

Antibiotics (mg/litre)	Butzler SR85	Blaser-Wang SR98	CCDA SR155	Preston SR117	Skirrow SR69	Karmali SR167	CAT SR174
Amphotericin B	–	2	10	–	–	–	10
Bacitracin	25,000*	–	–	–	–	–	–
Cephalothin	–	15	–	–	–	–	–
Cefazolin	15	–	–	–	–	32	–
Cefoperazone	–	–	32	–	–	–	8
Colistin	10,000*	–	–	5,000*	–	–	–
Cycloheximide	50	–	–	100	–	100	–
Novobiocin	5	–	–	–	–	–	–
Polymyxin	–	2,500*	–	–	2,500	–	–
Rifampicin	–	–	–	10	–	–	–
Trimethoprim	–	5	–	10	5	–	–
Vancomycin	–	10	–	–	10	20	–
Teicoplanin	–	–	–	–	–	–	4

*IU/L
CCDA – Modified Charcoal Cefoperazone Desoxycholate Agar (Blood-free medium)

Gun-Monro et al.[2] carried out a laboratory and clinical evaluation of the various selective isolation media for thermophilic campylobacters. Summary tables of their findings for the above antibiotic supplements are shown.

Table 1. Recovery of 70 *C. jejuni* strains on five selective media.

Medium	Colony Count	P value[b]
Blood agar control	7.95 ± 0.36	
Skirrow	7.82 ± 0.48	Ns[c]
Butzler	7.77 ± 0.51	<0.05
Blaser-Wang	7.70 ± 0.56	<0.05
Preston	7.76 ± 0.52	<0.05
Modified CCDA	7.91 ± 0.36	NS

Table 2. Isolation of *C. jejuni* from 70 simulated positive faeces samples.

Medium	24h	48h
Blood agar control	69 (99)	70 (100)
Skirrow	39 (56)	67 (96)
Butzler	38 (54)	60 (86)
Blaser-Wang	17 (24)	31 (41)
Preston	32 (46)	64 (91)
Modified CCDA	61 (87)	69 (99)

[a]Log_{10} mean colony counts ± standard deviation.
[b]significance determined by Student's t test for unpaired samples.
[c]NS, Not significant.

Table 3. Suppression of faecal flora from 70 simulated positive faecal samples.

Medium	No. (%) of plates with 75% reduction of faecal flora compared with control number (%) of strains isolated after incubation for: 24h	48h
Skirrow	46 (66)	38 (54)
Butzler	56 (80)	47 (67)
Blaser-Wang	28 (40)	15 (21)
Preston	58 (83)	50 (71)
Modified CCDA	64 (91)	59 (84)

The general conclusions reached by Gun-Munro et al. were confirmed by Griffiths and Ribeiro[3].

Enrichment broth cultures – the value of enrichment media for campylobacters is controversial[1] but in food and environmental studies enrichment may be essential[4]. Enrichment at 42°C[4,5] and cold enrichment at 4°C[6] have been reported. Where enrichment increases competitive flora, the use of membrane filters on the surface of the agar can help select campylobacters[7].

A comprehensive review of selective media for *Campylobacter* and *Arcobacter* species has been published in a special issue of International Journal of Food Microbiology.

Reference

Corry J.E.L., Post D.E., Colin P. and Laisney M.J. (1995). *Int. J. Food Microbiol. 26. 43–76.*

Laboratory growth environment

Atmosphere – members of the *Campylobacter* genus require a wide spectrum of atmospheres for optimum growth, ranging from complete anaerobiosis to

ambient air tolerance[8]. Most species, however, lie between these extremes and are micro-aerophilic. The Oxoid Campylobacter Gas Generating Kits (BR56 and BR60) ensure that the correct oxygen and carbon dioxide levels are produced for the optimum growth of these micro-aerophilic organisms. Alternatively use CampyGen CN025A or CN035A. CampyGen does not require the addition of water or a catalyst.

Temperature – the temperature range for incubation of *Campylobacter* species and related organisms varies from 15°C for *Arcobacter cryaerophilea* to 42°C for the thermophilic species. However, most strains have a considerable tolerance of growth temperatures around those required for optimum growth.

Helicobacter pylori – this organism is implicated as a cause of gastritis and peptic ulceration[9,10,11].

A specific selective culture medium, prepared from Helicobacter pylori Selective Supplement (Dent) SR147 and Columbia Blood Agar Base CM331 is required to isolate this organism from gastric biopsy specimens.

References

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CAMPYLOBACTER SELECTIVE MEDIA

For the isolation of campylobacters.

BASE MEDIA

BLOOD AGAR BASE NO. 2

Code: CM271

Formula	gm/litre
Proteose peptone	15.0
Liver digest	2.5
Yeast extract	5.0
Sodium chloride	5.0
Agar	12.0
pH 7.4 ± 0.2	

Directions
Suspend 20g in 500ml of distilled water, bring to the boil to dissolve completely. Mix and sterilise by autoclaving at 121°C for 15 minutes.

Or

COLUMBIA AGAR BASE

Code: CM331

Formula	gm/litre
Special peptone	25.0
Starch	1.0
Sodium chloride	5.0
Agar	10.0
pH 7.3 ± 0.2	

Directions
Suspend 19.5g in 500ml of distilled water. Boil to dissolve the medium completely. Sterilise by autoclaving at 121°C for 15 minutes.

CAMPYLOBACTER AGAR BASE

Code: CM689

Formula	gm/litre
'Lab-Lemco' powder	10.0
Peptone	10.0
Sodium chloride	5.0
Agar	12.0
pH 7.5 ± 0.2	

Directions
For preparation of Preston Campylobacter Selective Agar Base suspend 18.5g in 475ml of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C or below. Aseptically add 25ml of Lysed Horse Blood SR48.

See Campylobacter Selective Agar (Preston) for further information.

CAMPYLOBACTER SELECTIVE SUPPLEMENTS

Code: SR69 (Skirrow)
Code: SR85 (Butzler)
Code: SR98 (Blaser-Wang)
Code: SR117 (Preston)

Freeze-dried antibiotic supplements for the isolation of campylobacters.

CAMPYLOBACTER SELECTIVE SUPPLEMENT (SKIRROW)

Code: SR69 (Skirrow)

Vial contents (each vial is sufficient for 500ml of medium)

Vancomycin	5mg
Polymyxin	1,250 IU
Trimethoprim	2.5mg

Directions
To rehydrate the contents of the vial, add aseptically 2ml of sterile distilled water and turn end-over-end to dissolve. Avoid frothing the solution.

Add the contents of one vial to 500ml of sterile blood agar, cooled to 50–55°C, prepared from Oxoid Columbia Agar CM331 or Blood Agar Base No.2 CM271, with 5–7% lysed defibrinated horse blood. Mix gently and pour into sterile petri dishes.

Description
Campylobacter Selective Supplement SR69 is based on the formulation of Skirrow[1].

The antibiotic supplement is designed to be used at 42°C for optimum selective effect.

Reference
1 Skirrow M. B. (1977) *BMJ 2. 9–11.*

CAMPYLOBACTER SELECTIVE SUPPLEMENT (BUTZLER)

Code: SR85 (Butzler)

Vial contents (each vial is sufficient for 500ml of medium)

Bacitracin	12,500 IU
Cycloheximide	25mg
Colistin sulphate	5,000 IU
Cephazolin sodium	7.5mg
Novobiocin	2.5mg

Directions
To rehydrate the vial aseptically add 3ml of 50/50 ethanol/water and turn end-over-end to dissolve. Avoid frothing of the solution.

Add the contents of one vial to 500ml of sterile blood agar cooled to 50–55°C prepared from Oxoid Columbia Agar CM331, with 5–7% defibrinated horse or sheep blood. Mix gently and pour into sterile petri dishes.

Description
Campylobacter Selective Supplement SR85 is based on the formulation of Lauwers, De Boeck and Butzler[1].

Cephalothin (15µg/ml) has been replaced in the Oxoid Supplement by cephazolin (15µg/ml) as this has been found to be more inhibitory to *Pseudomonas* species[1].

This supplement differs from Skirrow Supplement SR69 by its selective action at 37°C. It therefore overcomes the need to use incubators at 42°C and it selectively isolates those strains of *Campylobacter* species that fail to grow at 42°C[2] (e.g. *C. fetus* sub.sp. *fetus*).

References
1 Lauwers S., De Boeck M. and Butzler J.P. (1978) *Lancet, 1. 604–605.*
2 Butzler J.P., Dekeyser P., Detrain M. and Dehaen F. (1973) *J. Pediat. 82. 493.*

CAMPYLOBACTER SELECTIVE SUPPLEMENT (BLASER-WANG)

Code: SR98 (Blaser-Wang)

Vial contents (each vial is sufficient for 500ml of medium)

Vancomycin	5mg
Polymyxin B	1,250 IU
Trimethoprim	2.5mg
Amphotericin B	1mg
Cephalothin	7.5mg

Directions
To rehydrate the contents of the vial, add aseptically 2ml of sterile distilled water and turn end-over-end to dissolve. Avoid frothing the solution.

Add the contents of one vial to 500ml of a sterile nutrient medium cooled to 50–55°C prepared from Columbia Agar Base CM331 or Blood Agar Base No.2 CM271, with 10% sheep blood or 5–7% laked horse blood SR48. Mix gently and pour into sterile petri dishes.

Description
Campylobacter Selective Supplement (Blaser-Wang) SR98 is based on the formulation of Skirrow[1], but with the addition of amphotericin B and cephalothin[2]. The inclusion of amphotericin B inhibits the growth of *Candida albicans* and cephalothin improves the selectivity of the supplement.

References
1 Skirrow M.B. (1977) *BMJ 2. 9–11.*
2 Blaser M.J., Hardesty H.L., Powers B. and Wang W.L.L. (1980) *J. Clin. Micro. 11. 309–313.*

CAMPYLOBACTER GROWTH SUPPLEMENT

Code: SR84

FBP Supplement for the enhanced growth and aerotolerance of campylobacter.

Vial contents (each vial is sufficient for 500ml of medium)

Sodium pyruvate	0.125g
Sodium metabisulphite	0.125g
Ferrous sulphate (hydrated salt)	0.125g

Directions
To rehydrate the contents of the vial, aseptically add 2ml of sterile distilled water and invert to dissolve. Avoid frothing of solution.

Add the contents of one vial to 500ml of a sterile nutrient medium cooled to 50–55°C prepared from Oxoid Columbia Agar CM331, Blood Agar Base No.2 CM271, or Campylobacter Agar Base CM689, with 5–7% lysed defibrinated horse or sheep blood, and the rehydrated contents of one vial of Campylobacter Antibiotic Supplement SR69, SR85 or SR98. Mix gently and pour aseptically into sterile petri dishes.

Description
Campylobacter species, which require less oxygen (i.e. microaerophilic) are inhibited at the normal atmospheric oxygen level. The optimum level of oxygen required for growth has been reported to be 6%[1]. This exacting level, together with a carbon

dioxide requirement, has made isolation of these organisms from human and animal sources a demanding procedure.

It is postulated that microaerophilic bacteria are more sensitive than other oxygen-dependent bacteria to toxic forms of oxygen (superoxide anions, peroxide, etc.) that occur in aerobic culture media. Compounds which enhance the aerotolerance of microaerophilic bacteria do so by quenching these toxic forms of oxygen[2].

Addition of 0.025% w/v each of ferrous sulphate, sodium metabisulphite and sodium pyruvate[3] has been found to increase the aerotolerance of *Campylobacter jejuni/coli*.

Addition of these compounds, presented together as Oxoid SR84 supplement to the culture medium will quench toxic oxygen compounds and will enable the oxygen sensitive strains of *Campylobacter* spp. to be more easily and rapidly isolated on a routine basis.

This means that the probability of isolating strains of *Campylobacter* spp. increases considerably. Those laboratories that can use candle jars only or use very approximate gas mixtures will particularly benefit from the addition of the growth supplement. FBP supplement protects medium from the formation of toxic compounds that may be formed because of exposure to light and air.

Studies have demonstrated the efficacy of FBP supplement in the repair of damaged Campylobacter cells in foods[4,5,6].

FBP is included in Preston enrichment broth[7,8] and selective enrichment broths based on Nutrient Broth No.2 specified by the Food and Drug Administration (FDA) for the detection of Campylobacter in foods, milk and water[9] and in Exeter medium specified by the Steering Group on the Microbiological Safety of Foods[10].

FBP supplement is incorporated in an improved medium for storage and transportation of thermophilic Campylobacters[11].

References

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9 F.D.A. Bacteriological Analytical Manual (1992) *7th Edition F.D.A. Washington D.C.*
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11 Rogol M., Schnaidman B., Katzenelson E. and Sechter I. (1990) *Eur. J. Clin. Microbiol. Inf. Dis. 9. 760–762.*

CAMPYLOBACTER AGAR BASE (KARMALI)

Code: CM935

A blood free selective medium for the isolation of Campylobacter jejuni and Campylobacter coli when incubated at 42°C.

Formula	gm/litre
Columbia Agar Base	39.0
Activated charcoal	4.0
Haematin	0.032
Final pH 7.4 ± 0.2	

CAMPYLOBACTER SELECTIVE SUPPLEMENT (KARMALI)

Code: SR167

Vial contents:

Sodium pyruvate	50.mg	(equivalent to 100mg/l)
Cefoperazone	16.mg	(equivalent to 32mg/l)
Vancomycin	10.mg	(equivalent to 20mg/l)
Cycloheximide	50.mg	(equivalent to 100mg/l)

Directions

Add 21.5 grams of Campylobacter Agar Base (Karmali) CM935 to 500ml of distilled water and bring to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add 1 vial of Campylobacter Selective Supplement (Karmali) SR167 reconstituted with 2ml of sterile distilled water. Mix well and pour into sterile petri dishes.

Description

Campylobacter Medium (Karmali) is based on the formulation described by Karmali et al[1] and is recommended for the isolation of *Campylobacter jejuni* and *Campylobacter coli* from clinical specimens.

The original Campylobacter Blood Free medium in the Oxoid product range contains sodium pyruvate in the agar base. Campylobacter Medium (Karmali) incorporates this ingredient into the selective supplement. The original medium also contains sodium desoxycholate for the inhibition of Gram positive organisms, whereas with Campylobacter Medium (Karmali) suppression of Gram positives is achieved by the inclusion of Vancomycin.

C. jejuni strains produce grey, moist, flat spreading colonies after 42 hour incubation at 42°C.

If plates are first examined after 24 hours incubation, read them immediately and quickly return them to a reduced oxygen atmosphere to ensure continued viability of the more oxygen-sensitive strains.

At 42°C selectivity is increased and growth is faster but non-thermophilic strains will not grow e.g. *C. fetus* subsp. *fetus*.

Colonies tend to swarm when initially isolated from clinical specimens.

Technique

1 Prepare Campylobacter Selective Medium (Karmali) plates as described in the directions for use.

2 Emulsify approximately 0.5g of the specimen in 5ml of sterile 0.1% peptone water to form an 1:10 dilution.

3 Inoculate on to selective medium with cotton tipped swabs so that single isolated colonies are formed.

4 Incubate the plates in an atmosphere consisting of approximately 5–6% oxygen, 10% carbon dioxide and 84–85% nitrogen for 48 hours at 42°C. This can best be achieved by using the Oxoid Gas Generating Kit for Campylobacters BR56 in conjunction with the Oxoid Anaerobic Jar and an active catalyst BR42. For jars of smaller capacity (2.5 litres) use the Oxoid Gas Generating Kit for Campylobacters BR60. Alternatively use CampyGen CN025A or CN035A. CampyGen does not require the addition of water or a catalyst.

5 Examine the plates and confirm the typical colonies as *Campylobacter jejuni* or *Campylobacter coli.* A simple schema for differentiating *Campylobacter* species is described by Skirrow and Benjamin[2].

Storage conditions and Shelf life
Campylobacter Agar Base (Karmali) should be stored tightly capped in the original container in a cool, dry place away from bright light. When stored as directed the medium will remain stable until the expiry date printed on the label.

Quality Control
Incubation at 42°C

Campylobacter jejuni ATCC® 29428	Growth
Escherichia coli ATCC® 25922	Partial to complete inhibition

Precautions
Campylobacter Selective Supplement (Karmali) contains cycloheximide and is toxic if swallowed, inhaled or comes into contact with the skin. As a precaution, when handling wear gloves and eye/face protection.

References

1 Karmali M.A., Simor A.E., Roscoe M., Fleming P.C, Smith S.S. and Lane J. (1986) *J.Clin.Micro. 23. 456–459.*

2 Skirrow M.B. and Benjamin J. (1980) *J.Clin.Path. 33.1122.*

CAMPYLOBACTER SELECTIVE AGAR (PRESTON)

A selective medium which when prepared from Campylobacter Agar Base CM689, Preston Campylobacter Selective Supplement SR117 and Lysed Horse Blood SR48 can be used for the selective isolation of Campylobacter jejuni and C. coli from human, animal, avian and environmental specimens.

CAMPYLOBACTER AGAR BASE

Code: CM689

Formula	gm/litre
'Lab-Lemco' powder	10.0
Peptone	10.0
Sodium chloride	5.0
Agar	12.0
pH 7.5 ± 0.2	

CAMPYLOBACTER SELECTIVE SUPPLEMENT (PRESTON)

Code: SR117

Vial contents (each vial is sufficient for 500ml of medium)

Polymyxin B	2,500IU
Rifampicin	5mg
Trimethoprim	5mg
Cycloheximide	50mg

Directions (to prepare Preston Campylobacter Selective Agar)
Suspend 18.5g of Campylobacter Agar Base (CM689) in 475ml of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add 25ml of Lysed Horse Blood SR48, and 1 vial of Preston Campylobacter Selective Supplement SR117 reconstituted with 2ml of 50/50 Acetone/sterile distilled water. Mix well and pour into sterile petri dishes.

Directions (to prepare Preston Campylobacter Selective Enrichment Broth)
Dissolve 12.5g of Nutrient Broth No.2 CM67 in 475ml of distilled water and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C or below. Aseptically add 25ml of Lysed Horse Blood SR48, 1 vial of Preston Campylobacter Selective Supplement SR117 and 1 vial of Campylobacter Growth Supplement SR84 both reconstituted as directed. Aseptically dispense 5ml volumes in sterile small screw-capped bottles. The Selective Enrichment Broth may be stored for up to 7 days at 4°C.

It is essential that the head space above the liquid should be as small as possible to ensure microaerobic conditions.

Description
The Preston Campylobacter Selective Agar is based on the formulation described by Bolton and Robertson[1]. This medium was specifically formulated to be suitable for isolation of *Campylobacter* species from all types of specimens (human, animal, avian and environmental).

In comparative studies[2] of the selective media of Skirrow, Butzler, Blaser, Campy-BAP and Preston, the Preston medium was found to give the maximum isolation rate of *Campylobacter* species from all types of specimens tested and also to be the most selective.

Oxoid Campylobacter Agar Base has been prepared from materials described by Bolton and Robertson[1]. It is suitable as a basal medium for the selective supplements of Blaser-Wang, Skirrow and Butzler.

Preston Campylobacter Selective Supplement SR117 can also be used to prepare Preston Campylobacter Selective Enrichment Broth[2].

The selective enrichment technique is recommended for specimens and food samples that are expected to be heavily contaminated and/or carry small numbers of viable colony forming units. The Preston Campylobacter Selective Enrichment Broth which is supplemented with the growth supplement SR84, made to the formulation of George et al.[3] effectively quenches toxic compounds which may form on exposure of the medium to light and air[4].

Technique

Direct Selective Plating Method

1 Prepare the Preston Campylobacter Selective Agar as directed from CM689, SR117 and Lysed Blood.
2 Emulsify the specimen under test in 2ml of 0.1% peptone water.
3 Inoculate onto the selective medium with cotton tipped swabs so that single isolated colonies are formed.
4 Incubate the plates in an atmosphere consisting of approximately 5–6% oxygen, 10% carbon dioxide and 84–85% nitrogen for 24–48 hours* at 42°C. This can best be achieved by using the Oxoid Gas Generating Kit for Campylobacters BR56 in conjunction with the Oxoid Anaerobic Jar and an active catalyst. For jars of smaller capacity (2.5 litres) use the Oxoid Gas Generating Kit for Campylobacters BR60. Alternatively use CampyGen CN025A or CN035A. CampyGen does not require the addition of water or a catalyst.
5 Examine the plates and confirm the typical colonies as *Campylobacter jejuni* or *C. coli* by the standard methods.

* When few *Campylobacter* colony forming units are present 48 hours incubation is necessary.

Selective Enrichment Broth Technique

1 Prepare the Preston Selective Enrichment Broth as directed from CM67, SR117, SR84 and Lysed Blood.
2 Emulsify the specimen under test in the selective enrichment broth.
3 Incubate the broth aerobically at 42°C for 24 hours.
4 Subculture on to Preston Campylobacter Selective Agar or Campylobacter Blood-Free Selective Agar.

CAMPYLOBACTER TRANSPORT MEDIUM

Campylobacter Selective Supplement (Preston) is incorporated in an improved medium for storage and transportation of thermophilic Campylobacters[5].

References

1 Bolton F.J. and Robertson L. (1982) *J. Clin. Pathol. 35. 462–467.*
2 Bolton F.J., Coates D., Hinchliffe P.M. and Robertson L. (1983) *J. Clin. Pathol. 36. 78–83.*
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4 Bolton F.J., Coates D. and Hutchinson D.N. (1984) *J. Appl. Bact. 56. 151–157.*
5 Rogol M., Schnaidman B., Katzenelso E. and Sechter I. (1990) *Eur. J. Clin. Microbiol. Inf. Dis. 9. 760–762.*

CAMPYLOBACTER BLOOD-FREE SELECTIVE MEDIUM (MODIFIED CCDA-PRESTON)

A medium, which when prepared from Campylobacter Blood-Free Selective Agar Base CM739 and CCDA Selective Supplement SR155, can be used for the isolation of Campylobacter jejuni, C. coli and C. laridis.

CAMPYLOBACTER BLOOD-FREE SELECTIVE AGAR BASE

Code: CM739

Formula	gm/litre
Nutrient Broth No.2	25.0
Bacteriological charcoal	4.0
Casein hydrolysate	3.0
Sodium desoxycholate	1.0
Ferrous sulphate	0.25
Sodium pyruvate	0.25
Agar	12.0
pH 7.4 $\pm$ 0.2	

CCDA SELECTIVE SUPPLEMENT

Code: SR155

An improved selective supplement for blood-free Campylobacter Agar.

Vial contents (each vial is sufficient to supplement 500ml of medium).

Cefoperazone	16mg	equivalent to 32mg/litre
Amphotericin B	5mg	equivalent to 10mg/litre

Directions

Suspend 22.75g of Campylobacter Blood-Free Selective Agar Base in 500ml of distilled water and bring to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add 1 vial of CCDA Selective Supplement SR155 reconstituted with 2ml of sterile distilled water. Mix well and pour into sterile petri dishes.

Description

Modified CCDA Medium is based on the original formulation described by Bolton et al[1] which was developed to replace blood with charcoal, ferrous sulphate and sodium pyruvate. Improved selectivity was achieved when cephazolin in the original formulation was replaced by cefoperazone as the selective agent[2]. More recent work has shown an increased isolation rate can be achieved if the plates are incubated at 37°C rather than 42°C[3].

Amphotericin B has been added to the formula to suppress the growth of yeast and fungal contaminants that may occur at 37°C.

Modified CCDA medium and Campy-BAP medium were equal in performance in a rapid colony-lift procedure for detection of thermophilic campylobacters in which membranes are blotted on agar cultures and then subjected to immunoassay[5].

In a study of healthy puppies and kittens for carriage of *Campylobacter* species[6], modified CCDA medium was found to be a suitable medium and more

productive for *C. upsaliensis* in this application than CAT medium. Modified CCDA medium has been confirmed as suitable for isolation of *Campylobacter* spp. from non-clinical samples following enrichment in Exeter broth[7].

The use of Campylobacter Blood-Free Medium is specified by the U.K. Ministry of Agriculture, Fisheries and Food (MAFF) in a validated method for isolation of Campylobacter from foods[4].

Technique

1 Prepare Campylobacter Blood-Free Selective Agar as described in the directions.
2 Emulsify approximately 0.5g of the specimen in 5ml of sterile 0.1% peptone water to form an approximate 1:10 dilution.
3 Inoculate onto the selective medium with cotton tipped swabs so that single isolated colonies are formed.
4 Incubate the plates in an atmosphere consisting of approximately 5–6% oxygen, 10% carbon dioxide and 84–85% nitrogen for 48 hours at 37°C. This can best be achieved by using the Oxoid Gas Generating Kit for Campylobacters (BR56) in conjunction with the Oxoid Anaerobic Jar and an active catalyst (BR42). For jars of smaller capacity (2.5 litres) use the Oxoid Gas Generating Kit for Campylobacters (BR60). Alternatively use CampyGen CN023A or CN035A which does not require the use of a catalyst or the addition of water.

The colonial morphology of campylobacters can be used as a guideline for identification to species level. *C. jejuni* strains produce grey, moist flat spreading colonies. Some strains may have a green hue or a dry appearance, with or without a metallic sheen. *C. coli* strains tend to be creamy-grey in colour, moist, slightly raised and often produce discrete colonies.

Colonies tend to swarm when initially isolated from clinical specimens.

Storage conditions and Shelf life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the selective supplement in the dark at 2–8°C and use before the expiry date on the label.

The prepared medium may be stored for up to 2 weeks at 2–8°C.

Quality Control

Incubation at 37°C for 48 hours.

Positive control:
Campylobacter jejuni ATCC® 29428

Negative control:
Escherichia coli ATCC® 25922

References

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CEFOPERAZONE, AMPHOTERICIN B, TEICOPLANIN SUPPLEMENT (CAT)

Code: SR174

A selective supplement for the isolation of thermophilic Campylobacter spp. and improved recovery of Campylobacter upsaliensis from faeces.

Vial contents (each vial is sufficient for 500ml of medium)

CAT Supplement	gm/litre
Cefoperazone	8.0
Teicoplanin	4.0
Amphotericin B	10.0

Directions

Aseptically add 4ml of sterile distilled water to the vial. Mix gently to resuspend the supplement.

Prepare 500ml of sterile Blood-Free Campylobacter Agar Base CM739 as directed. Cool to 50°C and aseptically add one vial of SR174E reconstituted as directed above.

Mix well and pour the resulting CAT medium into sterile petri dishes. Incubate cultures at 37°C for 48–72 hours in a microaerobic atmosphere.

Description

Because of the sensitivity of *C. upsaliensis* to a wide range of antibiotics, isolation of the organism from faeces using selective media has hitherto been difficult. The recommended isolation method uses a membrane filter culture technique on non-selective agar. This does not give good recovery from faeces containing less than 10^5 CFU/g[5] however, and is a technically demanding method which is relatively slow to perform.

CAT Supplement SR174 is based on the formulation described by Aspinall et al[7]. When added to Blood-Free Campylobacter Agar Base CM739 which contains charcoal, it gives good isolation of thermophilic *Campylobacter* spp., and makes the isolation of *C. upsaliensis* possible on a selective medium because CAT Supplement contains reduced levels of cefoperazone compared to other Campylobacter supplements. This inhibits most Enterobacteriaceae, but not enterococci. Teicoplanin is included to inhibit enterococci. Amphotericin B is added as an antifungal agent.

Further work confirmed the effectiveness of CAT medium as an alternative to membrane filtration culture for selective isolation of thermophilic campylobacters including *C. upsaliensis*[8].

Atabay, Corry and On[9] isolated a previously

unknown catalase-negative, urease-positive Campylobacter from cattle faeces using CAT medium. This organism could not be cultured on blood-free Campylobacter medium (CCDA).

A study in which the productivity of CAT medium, 2 blood-free media and semi-solid medium were compared, showed that CAT medium, used in parallel with membrane filtration on non-selective blood agar, is likely to be the most productive method for recovery of the greatest number of *Campylobacter* and *Arcobacter* species[10].

Storage conditions and Shelf life
CAT Supplement SR174 should be stored at 2°C–8°C in the dark. When stored as directed, the reagents remain stable until the stated expiry date shown on the packaging.

Quality Control
Positive control:

C. upsaliensis	NCTC 11926 (pale grey colonies)
C. jejuni	ATCC® 29428 (grey colonies)

Negative control:

Enterococcus faecalis	ATCC® 33186 (inhibited)

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CARY-BLAIR MEDIUM

Code: CM519
A transport medium for Gram negative and anaerobic organisms.

Formula	gm/litre
Disodium hydrogen phosphate	1.1
Sodium thioglycollate	1.5
Sodium chloride	5.0
Calcium chloride	0.09
Agar	5.6
pH 8.4 ± 0.2	

Directions
Suspend 13.3g in 1 litre of distilled water and bring gently to the boil to dissolve the agar. Distribute into small, screw-cap bottles and sterilise by immersing in free-steam for 15 minutes. Allow to cool and tighten the screw caps to prevent water loss.

Description
Oxoid Cary-Blair Medium is a transport medium for the collection and shipment of clinical specimens based on the formulation of Cary and Blair[1].

The low nutrient content of the medium and utilisation of phosphate as a buffering agent instead of sodium glycerophosphate, prevents bacterial overgrowth by *Escherichia coli*, *Citrobacter freundii* and *Klebsiella aerogenes*.

This sometimes happens when using Stuart Transport Medium because these organisms possess specific glycerophosphate dehydrogenases[2]. The low oxidation-reduction potential of the medium ensures bacterial survival over long periods[3].

Cary and Bair[1] reported recovery of cholera vibrios up to 22 days, salmonellae and shigellae after 49 days and *Yersinia pestis* up to 75 days storage at 28°C.

Cary-Blair Medium is particularly suitable in field epidemiological surveys for *Vibrio parahaemolyticus*, especially where rectal swabs are to be transported to a central diagnostic laboratory[4,5]. Survival of *V. parahaemolyticus* in Cary-Blair medium has been reported after a 35-day period at a temperature of 70–80°F[6].

The medium can be modified to improve the transport and survival of *Campylobacter* species.

(i) by the addition of 1% w/v sodium pyruvate (10g/1itre) to the formula[7].
(ii) reducing the agar content from 5g to 1.6g per litre[8].

For the transport of fastidious anaerobic bacteria the medium may be prepared as directed and filled into long narrow screw-capped tubes[9]. It may also be prepared as a pre-reduced anaerobic sterilised medium (PRAS)[10]. Methods of producing PRAS media are described by Holdeman and Moore[11].

Technique
Use sterile, cotton-tipped swabs on wooden sticks to collect the specimen. Push the swabs down one third of the medium depth and cut the stick. Screw the cap firmly on the bottle.

Label the bottle and send it to the laboratory without delay.

The recovery of *Shigella* species is higher when the transport medium is held at 4°C or frozen[12].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

The prepared medium should be stored away from light at 2–8°C or at room temperature (22–25°C) up to 19 months[13].

Quality Control
Positive control:
Shigella sonnei ATCC® 25931
Vibrio parahaemolyticus NCTC 11344

Negative control:
Uninoculated medium.

Precautions
The medium should not be incubated to check sterility, prior to use. This should be carried out on separate quality control samples.

The medium can maintain the viability of fastidious organisms for transport purposes but it should not be used as a storage or enrichment medium.

The results obtained from the medium are dependent on the quality of the specimen material. Commensal anaerobic organisms may overgrow in the medium and cause misleading results.

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CHARCOAL AGAR

Code: CM119

A medium for the cultivation and isolation of Bordetella pertussis and Haemophilus influenzae.

Formula	gm/litre
'Lab-Lemco' powder	10.0
Peptone	10.0
Starch	10.0
Charcoal bacteriological	4.0
Sodium chloride	5.0
Nicotinic acid	0.001
Agar	12.0
pH 7.4 $\pm$ 0.2	

Directions
Suspend 51g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C, add 10% of defibrinated blood and mix gently. The medium is made selective for the isolation of *Bordetella pertussis* and *B. parapertussis* by the addition of Bordetella Selective Supplement SR82.

To one vial add 2ml of sterile distilled water and dissolve the contents completely. Add this solution to 500ml of sterile, molten Charcoal Agar CM119, cooled to 50°C, together with 10% v/v defibrinated horse blood SR50. Mix well before pouring into sterile petri dishes.

For *Haemophilus influenzae*, omit the selective agents and convert to 'chocolate' agar.

Transport Medium for *B. pertussis*
The vial contents may be added to 500ml of half-strength Charcoal Agar + 10% v/v defibrinated horse blood SR50 for use as a transport medium for *B. pertussis.*

Description
Charcoal Agar CM119 was developed by Oxoid to provide a non-blood containing medium for the cultivation of *Bordetella pertussis* and *Haemophilus influenzae*. Proom[1] showed that nicotinic acid was an essential growth factor for the bordetellae. Ensminger et al.[2] used a charcoal medium for the growth of *B. pertussis* in vaccine production and found that the medium could replace Bordet-Genou. Mishulow et al.[3] used charcoal agar for *B. pertussis* cultivation.

H. influenzae is cultivated on the medium containing 10% 'chocolated' blood but no antibiotics. The inoculated plate is incubated for 2 to 3 days at 37°C. The colonies are usually small, transparent and droplet-like, but some transformation to the 'rough' type colony may occur. Species differentiation is performed by examination of the need for X and V growth factors, on Blood Agar Base CM55.

The greatest problem in the isolation of *Bordetella* species from naso-pharyngeal secretions is the suppression of unwanted flora during the long incubation period on very nutritious media.

Fleming's first *in vitro* demonstration of penicillin was to show that it could help isolate *B. pertussis* on media[4]. Lacey[5] confirmed this but found that the penicillin-resistant flora still caused problems. He supplemented penicillin with 2µg/ml 4,4' diamidino-diphenylamine dihydrochloride (M & B 938) thereby increasing the selectivity of this medium.

Broome et al.[6] found methicillin to be superior to penicillin in suppressing unwanted naso-pharyngeal flora but the earlier publication of Sutcliffe and Abbott[7] where cephalexin (40µg/ml) was shown to be superior to penicillin, has proved to be the most significant advance.

The benefits of cephalexin as a selective agent for *B. pertussis* have been confirmed[8,9,10,11]. The ability to recover stressed cells and the much longer shelf life (6–8 weeks) are added benefits to its superiority at suppressing unwanted naso-pharyngeal growth.

Regan and Lowe[8] showed that half-strength Oxoid Charcoal Agar, supplemented with 40µg/ml cephalexin SR82 v/v lysed, defibrinated horse blood was an excellent enrichment and transport medium.

The efficacy of this transport medium has been confirmed by other workers[12].

Technique
The following technique for the laboratory diagnosis of *Pertussis* is recommended[11].

1 Collect pernasal swabs in the early stage of the illness and place in tubes of half-strength Charcoal Agar supplemented with 10% v/v lysed, defibrinated horse blood and 40µg/ml cephalexin.
2 Generously inoculate the swabs on to thick layers of Charcoal Agar CM119 containing 10% v/v defibrinated horse blood and 40µg/ml cephalexin (SR82).

   A non-selective medium in which the cephalexin is omitted may be used in addition.
3 Perform direct fluorescent antibody (DFA) tests on the secretions, using *B. pertussis* and *B. parapertussis*-conjugated antisera, to help make an earlier diagnosis.
4 Replace the swabs in the original transport medium and hold at room temperature. If the culture plates become overgrown with commensal flora or fungi, use the swabs to inoculate fresh plates of medium.
5 Incubate the plates at 35°C in a moist atmosphere (60–70% humidity) for up to six days. Examine the plates after 40 hours incubation and twice-daily thereafter.
6 Look for small, shiny, greyish-white, round convex colonies. Suspicious colonies should be Gram stained, using a two-minute safranin counterstain. Some pleomorphic cells may be seen, caused by the cephalexin in the selective medium.
7 Confirm the identification with DFA tests on the suspicious colonies.

Precautions
Stuart's transport medium or similar formulation media should not be used for Bordetella-containing specimens[13].

Two pernasal swabs should be taken from each patient, one through each nostril[14].

Make sure the charcoal remains in suspension when dispensing the medium by gently swirling the flask.

Lysed horse blood is used in the transport medium but whole blood is used in the isolation medium.

Most naso-pharyngeal flora are inhibited by cephalexin but *Pseudomonas aeruginosa* and some fungi may grow through. Amphotericin B can be added (12mg/ml) as an antifungal agent to prevent the growth of filamentous fungi. However, this level of amphotericin B can be inhibitory to *B. pertussis* and should not be used routinely.

METRONIDAZOLE SUSCEPTIBILITY TEST FOR

Helicobacter pylori

Charcoal agar supplemented with a concentrate of essential growth factors has been reported to be a reliable testing medium for determining metronidazole resistance in *Helicobacter pylori*[15].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium at 2–8°C.

Quality Control
With Cephalexin:
Positive control:
Bordetella pertussis ATCC® 8467
Bordetella parapertussis ATCC® 10521

Negative control:
Staphylococcus aureus ATCC® 25923
Klebsiella pneumoniae ATCC® 13883

Without Antibiotics:
Positive control:
Haemophilus influenzae ATCC® 35056

Negative control:
Uninoculated medium

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CHINA BLUE LACTOSE AGAR

Code: CM209

A standard, non-inhibitory solid medium for enumeration and differentiation of bacteria in dairy products.

Formula	gm/litre
Peptone	5.0
'Lab-Lemco' powder	3.0
Lactose	10.0
Sodium chloride	5.0
China blue	q.s.
Agar	12.0
pH 7.0 ± 0.2	

Directions
Suspend 35g in 1 litre of distilled water. Boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
China Blue Lactose Agar was formulated by Brandl and Sobeck-Skal[1]. A standard, non-inhibitory solid medium for the differentiation and enumeration of bacteria in milk, proposed by the Methodenkommission für Milchwirtschaft[2]. The china blue serves as a pH indicator, to differentiate between lactose fermenters and non-lactose fermenters, but does not suppress the growth of cocci; therefore this medium may be used for the detection of streptococci and staphylococci as well as the coli-aerogenes group.

Plates may be streak-inoculated or decimal dilutions of milk may be added to the molten, cooled medium in a pour-plate technique.

After 18 hours incubation at 35°C colony appearances are:

Colour	Micro-organisms
Blue	Lactose fermenters e.g. *Escherichia coli* and coliform bacteria: 3–4mm diameter. Staphylococci: 1mm diameter Streptococci: 0.5mm diameter.
Colourless	Non-lactose fermenters e.g. *Salmonella, Serratia, Proteus* species and others.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Enterococcus faecalis ATCC® 29212
Escherichia coli ATCC® 25922

Negative control:
Uninoculated medium.

Precautions
It is important to remember that Gram positive and negative cocci and bacilli can grow on this medium. Always confirm the organism morphology and Gram reaction.

References

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CHOLERA MEDIUM TCBS

Code: CM333

A selective isolation medium for pathogenic vibrios.

Formula	gm/litre
Yeast extract	5.0
Bacteriological peptone	10.0
Sodium thiosulphate	10.0
Sodium citrate	10.0
Ox Bile	8.0
Sucrose	20.0
Sodium chloride	10.0
Ferric citrate	1.0
Bromothymol blue	0.04
Thymol blue	0.04
Agar	14.0
pH 8.6 ± 0.2	

Directions
Suspend 88 grams in 1 litre of distilled water. Boil to dissolve the medium completely. DO NOT AUTOCLAVE.

Pour plates without further heating and dry before use.

Description
Kobayashi, Enomoto, Sakazaki and Kuwahara[1] developed TCBS media from the selective isolation agar of Nakanishi[2].

The Oxoid TCBS medium conforms to the formulation of Kobayashi et al., except that it contains specially processed ox bile, free from the defects noted by Nakanishi and Kobayashi.

The complexity of the composition of this medium means that uniformity of growth is a difficult standard to maintain. Several investigations have shown variation between batches of TCBS Medium made by different companies[3,4,5,6].

Quality control by the manufacturers of this medium is especially important because satisfactory inhibition of normal gut flora and lack of inhibition of certain *Vibrio* species is very critical. West et al.[7] showed that Oxoid TCBS Medium came closest to their criteria for a satisfactory product.

WHO has established a minimum acceptable guideline for the recovery of *Vibrio* species on TCBS Medium[8].

The Oxoid medium is suitable for the growth of *Vibrio cholerae*, *V. parahaemolyticus*, and most other vibrios[9].

Most of the Enterobacteriaceae encountered in faeces are totally suppressed for at least 24 hours. Slight growth of *Proteus* species and *Strept. faecalis* may occur but the colonies are easily distinguished from vibrio colonies.

Oxoid TCBS Medium is complete and requires no additives or aseptic additions of blood. It therefore shows a considerable advantage over Lauryl Sulphate Tellurite Agar which requires further additions after sterilisation. Apart from this convenience factor, it also possesses superior growth characteristics for *Vibrio* species, compared with tellurite media. Whilst inhibiting non-vibrios, it promotes rapid growth of

pathogenic vibrios after overnight incubation at 35°C. For the isolation of other vibrios from environmental samples, incubation at lower temperatures, 20–30°C is needed.

Colonial appearance of organisms on TCBS Medium
24 hours incubation at 35°C.

Organisms	Colonies
V. cholera and El Tor type	Yellow, flat, 2–3mm diameter
V. parahaemolyticus	Blue-green, 3–5mm diameter
V. alginolyticus	Yellow, 3–5mm diameter
V. metschnikovii[10]	Yellow, 3–4mm diameter
V. fluvialis[11]	Yellow, 2–3mm diameter
V. vulnificus[12]	Blue-green, 2–3mm diameter
V. mimicus[13]	Blue-green, 2–3mm diameter
Enterococcus species	Yellow, 1mm diameter
Proteus species	Yellow-green, 1mm diameter
Pseudomonas species	Blue-green, 1mm diameter

Some strains of *Aeromonas hydrophila* grow producing yellow colonies but *Plesimonas shigelloides* does not usually grow well on TCBS.

Technique
Streak the faeces or a subculture from enrichment media, e.g. Alkaline Peptone Water across the surface of Oxoid TCBS Cholera Medium and incubate for 18–24 hours at 35°C for clinical specimens or lower temperature for environmental samples.

Cultures grown on TCBS should be examined quickly after removal from an incubator as the yellow colonies of cultures of vibrios e.g. *V. cholerae* may revert to a green colour when left at room temperature[9].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Vibrio furnissii NCTC 11218 (a non-pathogenic strain[6])

Negative control:
Escherichia coli ATCC® 25922

Precautions
The identification of the various *Vibrio* species on TCBS Medium is presumptive and further tests are required for confirmation.

Yellow colonies on TCBS Medium will give unsatisfactory oxidase reactions.

Colonies taken from TCBS Medium are 'sticky' and react poorly in slide agglutination tests. Subculture to nutrient agar is required before slide agglutination tests can be carried out.

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CHROMOGENIC E. COLI/ COLIFORM MEDIUM

Code: CM956

A chromogenic medium to aid differentiation between Escherichia coli and other coliforms in cultures produced from food and environmental samples.

Formula	gm/litre
Chromogenic mix	20.3
Agar	15.0
Yeast extract	3.0
Peptone	5.0
Lactose	2.5
Sodium chloride	5.0
Di-sodium hydrogen phosphate	3.5
Potassium di-hydrogen phosphate	1.5
Neutral red	0.03

Directions
Suspend 55.8g of Chromogenic Escherichia coli/ Coliform Medium in 1 litre of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Mix well and pour into sterile petri dishes.

Dry the surface of the medium in the prepared plates. Prepare the food sample by diluting 1 in 5 or 1 in 10 (as appropriate) with 0.1% (w/v) sterile Peptone

Water CM9, and homogenise in a stomacher or laboratory blender. Pipette 0.5ml or 1.0ml (as appropriate) of the homogenate on to the plate and spread over the surface with a sterile glass spreader. Incubate plates for 18–24 hours at 37°C.

Multiply the number of purple colonies by the dilution factor and express the result as the number of *E. coli* per gram of food.

Description
Chromogenic Escherichia coli/Coliform Medium CM956 is a differential agar which provides presumptive identification of *E. coli* and coliforms in food and environmental samples.

The agar base uses two enzyme substrates to improve differentiation between *E. coli* and other coliforms.

One chromogen allows specific detection of *E. coli* through the formation of purple colonies. This substrate is cleaved by the enzyme glucuronidase which is produced by approximately 97% of *E. coli* strains.

The other chromogen is cleaved by the enzyme galactosidase, which is produced by the majority of coliforms, resulting in rose/pink colonies.

Storage conditions and Shelf life
Chromogenic Escherichia coli/Coliform Medium CM956 must be stored tightly capped in the original container at 10°C–25°C. When stored as directed, the medium will remain stable until the expiry date printed on the bottle.

Quality Control
Positive control:
Escherichia coli ATCC® 25922 – purple colonies
Klebsiella pneumoniae ATCC® 11228 – rose/pink colonies

Negative control:
Pseudomonas aeruginosa ATCC® 27853 – straw colonies

Precautions
Chromogenic Escherichia coli/Coliform Medium CM956 must only be used for *in vitro* diagnostic purposes.

Do not use beyond the stated expiry date, or if the product is caked, discoloured or shows any sign of deterioration.

Wear dust mask when handling the dehydrated product. Avoid contact with eyes.

References

1. Kilian M. and Bulow P. (1976). *Acta. Pathol. Microbiol. Scand. Sect. B 84: 245–251.*
2. Kilian M. and Bulow P. (1979). *Acta. Pathol. Microbiol. Scand. Sect. B 87: 271–276.*
3. Frampton E.W., Restaino L. and Blaszko N. (1988). *J. Food Prot. 51(5): 402–404.*

CHROMOGENIC URINARY TRACT INFECTION (UTI) MEDIUM

Code: CM949

A chromogenic medium for the presumptive identification and differentiation of all the main micro-organisms that cause urinary tract infections (UTIs).

Formula	gm/litre
Peptone	15.0
Chromogenic mix	26.3
Agar	15.0
Final pH 6.8 ± 0.2	

Directions
Suspend 56.3 grams of Chromogenic UTI Medium in 1 litre of distilled water, mix well and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and mix well before pouring plates.

Description
Chromogenic UTI Medium CM949 contains two specific chromogenic substrates which are cleaved by enzymes produced by *Enterococcus* spp., *E. coli* and coliforms. In addition, it contains phenylalanine and tryptophan which provide an indication of tryptophan deaminase activity, indicating the presence of *Proteus* spp., *Morganella* spp. and *Providencia* spp. It is based on electrolyte deficient CLED Medium which provides a valuable non-inhibitory diagnostic agar for plate culture of other urinary organisms, whilst preventing the swarming of *Proteus* spp.

One chromogen, X-Gluc, is targeted towards β-glucosidase, and allows the specific detection of enterococci through the formation of blue colonies.

The other chromogen, Red-Gal, is cleaved by the enzyme β-D galactosidase which is produced by *E. coli*, resulting in pink colonies. Any uncertainty in identification may be resolved by removing suspect *E. coli* colonies from the plate and performing an indole test using DMACA reagent.

Cleavage of both chromogens occurs in the presence of coliforms, resulting in purple colonies.

The medium also contains tryptophan which acts as an indicator of tryptophan deaminase activity, resulting in colonies of *Proteus*, *Morganella* and *Providencia* spp. appearing brown.

Table of typical colour reactions

Organism	β-D galactosidase	β-glucosidase	Tryptophan deaminase TDA	Colony colour
Enterococci		+		Blue
E. coli	+			Pink
Coliforms	+	+		Purple
Proteus/ Morganella and *Providencia*			+	Brown
Pseudomonas				Fluoresce
Staphylococcus				Normal pigmentation

It should be noted that organisms with atypical enzyme patterns may give anomalous reactions. For example, in a trial[6], over 45% of Enterobacter cloacae were shown to lack β-glucosidase, resulting in pink colonies which were indistinguishable from *E. coli*. In such cases, an indole test can be performed using DMACA indole (do not use Kovac's as the colour of the *E. coli* colonies may interfere with the red colour of a positive indole test). The reagent should not be applied directly to the plate, but the test should be performed on filter paper. This test will distinguish between *E. coli* and Enterobacter, and also between *Proteus mirabilis* and other species.

Storage conditions and Shelf life
Chromogenic UTI Medium CM949 must be stored tightly capped in the original container at room temperature. When stored as directed, the medium will remain stable until the expiry date printed on the bottle.

Quality Control

		Colour reaction
Escherichia coli	ATCC® 25922	Pink
Klebsiella pneumoniae	ATCC® 29665	Purple
Enterococcus faecalis	ATCC® 29212	Blue
Proteus mirabilis	ATCC® 10975	Brown
Staphylococcus aureus	ATCC® 25923	Typical appearance

Precautions
Chromogenic UTI Medium CM949 must only be used for *in vitro* diagnostic purposes.

Do not use beyond the stated expiry date, or if the product is caked, discoloured or shows any sign of deterioration.

Wear dust mask when handling the dehydrated product. Avoid contact with eyes.

References

1 Pezzlo, M. (1998). *Clinical Microbiology Reviews 1: 268–280.*
2 Wilkie, M.E., Almond, M.K., Marsh, F.P. (1992). *British Medical Journal 305: 1137–1141.*
3 Friedman, M.P. et al. (1991). *Journal of Clinical Microbiology 29: 2385–2389.*
4 Murray, P., Traynor, P., Hopson, D. (1992). *Journal of Clinical Microbiology 30: 1600–1601.*
5 Soriano, F., Ponte, C. (1992). *Journal of Clinical Microbiology 30: 3033–3034.*
6 Data on file.

CLAUSEN MEDIUM

DITHIONITE-THIOGLYCOLLATE (HS-T) BROTH

Code: CM353

The Nordic Pharmacopoeia Board have recommended this new medium, containing neutralising compounds and supplementary minerals, for sterility testing.

Formula	gm/litre
Tryptone	15.0
Yeast extract	6.0
Soya peptone	3.0
Glucose	6.0
Sodium chloride	2.5
Dipotassium hydrogen phosphate	2.0
Sodium citrate	1.0
L-cystine	0.5
L-asparagine	1.25
Sodium dithionite	0.4
Sodium thioglycollate	0.5
Lecithin	0.3
Magnesium sulphate .7H_2O	0.4
Calcium chloride .2H_2O	0.004
Cobalt sulphate .7H_2O	0.001
Cupric sulphate .5H_2O	0.001
Ferrous sulphate .7H_2O	0.001
Zinc sulphate .7H_2O	0.001
Manganese chloride .4H_2O	0.002
Resazurin	0.001
Agar	0.75
pH 7.1 $\pm$ 0.2	

Directions
Suspend 40g in a solution composed of Tween 80 (polyethylene sorbitan mono-oleate) 3g: glycerol 5g and distilled water 1 litre. Bring to the boil to dissolve completely.

Distribute into tubes or bottles and sterilise by autoclaving at 121°C for 15 minutes.

THE MEDIUM MUST NOT BE RE-STERILISED.

Description
Dithionite-thioglycollate (HS-T) Broth was developed by Clausen in Oslo University and has been recommended for sterility testing by the Nordic Pharmacopoeia Board. The problems of sterility testing by selecting random samples is recognised by the Board and they refer to the process as the Microbial-Contamination Test. The standard microbial-contamination test is designed solely to establish that the number of non-sterile units, if any, in a batch is below a certain level.

The following description of the Standard Microbial-Contamination Test has been abridged from the detailed description published as an addendum in the Nordiska Farmakopenämnden.

The tests must be performed with all precautions taken to prevent laboratory contamination occurring more than once at the most in every 100 tests. The use of laminar air-flow cabinets is recommended. Tests are to be made by qualified and experienced staff and the efficiency of the methods used must be checked at regular intervals.

A random sample of sufficient quantity to be representative of the whole bulk, should be examined.

Two methods of detecting non-sterile units may be used in the microbial-contamination test.

Membrane Filter Method
The test substance is dissolved or suspended in 200ml of 0.1% w/v sterile (pH 7.0–7.2) CM9 and immediately filtered through one or more membrane filters (average pore diameter 0.45μ or less).

Each filter is then washed three times, by passing 100ml volumes of peptone solution through the membrane.

After filtration the membranes are transferred to tubes of media, containing at least 15ml of Clausen Medium and tubes of Tryptone Soya Broth (soybean-casein digest medium) CM129. If only one filter is used, this is divided into two and the two halves placed in separate tubes.

Tubes of Clausen Medium are incubated for at least 14 days at 30–32°C.

Tubes of Tryptone Soya Broth (soybean-casein digest medium) are incubated for at least 14 days at 20–25°C.

Dilution Method
From each sample 1.0ml of material or suspension is transferred to each of at least 10 tubes containing a minimum quantity of 15ml of Clausen Medium.

One half of the number of tubes is incubated at 30–32°C for at least 14 days and the other half at 20–25°C for the same time.

If the medium becomes turbid on incubation, subcultures must be taken as soon as possible. Subcultures must also be taken after normal incubation and observed for a further period of 14 days.

Assessment Of The Results
The standard microbial contamination test is passed if growth is not observed in any of the tubes. If growth is observed, the test may be repeated with twice the number of samples. The test is then passed if no growth is observed in any of these tubes. Growth is diagnosed by the appearance of turbidity in fluid or semi-fluid media, by the formation of colonies on solid media, or by microscopy of culture samples.

Controls
Both methods of testing must be controlled for microbial inhibitors by adding a small inoculum of organisms (approximately 10 colony-forming bacteria) either to the tubes prepared in Method II or to peptone diluent, prior to filtration, in Method I.

If no growth occurs in the tubes containing the test organisms then the test must be repeated with the growth-inhibitory effect inactivated.

The test organisms recommended are:
Staphylococcus epidermidis
Clostridium sporogenes
Rhodotorula rubra

They are maintained on agar slants or deep agar stabs and the test inoculum is prepared from 24 hour cultures grown in Clausen Medium at 30–32°C. The *Rh. rubra* inoculum is prepared from a 48 hour culture grown in the same broth at 20–25°C.

Dithionite-Thioglycollate Broth was formulated by Clausen as a highly nutritious medium containing reducing agents and essential metals for the recovery of anaerobic spore-bearing organisms. It also contains lecithin and Tween 80 to overcome the effects of cationic agents which may show powerful bacteriostatic effects *in vitro*.

The broth should be prepared as directed and transferred to tubes or bottles in sufficient volumes (at least 15ml) for the standard microbial-contamination test and rapidly cooled to 20°C after sterilisation.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Prepared medium: The medium should be stored in a cool place (not at 4°C) away from the light, for a maximum period of one month.

Quality Control
Positive control:
see Control section

Negative control:
Uninoculated medium.

Precautions
The medium is yellowish in colour and almost clear. It turns pale-pink under aerobic conditions. The upper third only of the medium should be pink by the time it is to be used.

Bibliography
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Clausen O. G. (1973) *'A study of the growth-promoting properties of fluid and solid microbial-contamination test media on small numbers of micro-organisms.' 'Pharmaceutica Acta Helvetiae''48. 541–548.*
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CLED MEDIUM

Code: CM301

Recommended for diagnostic urinary bacteriology. The medium supports the growth of all urinary potential pathogens giving good colonial differentiation and clear diagnostic characteristics.

Formula	gm/litre
Peptone	4.0
'Lab-Lemco' powder	3.0
Tryptone	4.0
Lactose	10.0
L-cystine	0.128
Bromothymol blue	0.02
Agar	15.0
pH 7.3 ± 0.2	

Directions
Suspend 36.2g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring.

Description
A dehydrated Cystine-Lactose-Electrolyte Deficient (CLED) medium made to the formula described by Mackey and Sandys[1] as a modification for urinary bacteriology of the Electrolyte Deficient Medium developed by Sandys[2].

This medium is recommended for urinary bacteriology, supporting the growth of all urinary pathogens and giving good colonial differentiations and clear diagnostic characteristics. The presence of important contaminants such as diphtheroids, lactobacilli and micrococci is also clearly elicited, giving an indication of the degree of contamination.

In the laboratory CLED Medium provides a valuable non-inhibitory diagnostic agar for plate culture of urinary organisms. It is electrolyte deficient to prevent the swarming of *Proteus* species.

The medium has been used successfully in the Dip-inoculum Transport Medium technique (Mackey and Sandys[1,3]).

A variant of this technique has been described by Guttman and Naylor[4] who employed media-coated slides.

These techniques overcome false bacteriological results associated with delay in the transport of the specimens of urine to the laboratory and permit a clinically accurate routine differential viable count. They are, therefore, suitable for both general practitioner and hospital work including the screening of ante-natal specimens for symptomless bacteriuria.

For full details, the original papers should be consulted.

Growth Characteristics on CLED Agar (18 hours Incubation)

E. coli – yellow, opaque colonies with a slightly deeper coloured centre about 1.25mm diam. (Non-lactose fermenting strains – blue colonies.)

***Klebsiella* species –** extremely mucoid colonies varying in colour from yellow to whitish-blue.

***Proteus* species** – translucent blue colonies usually smaller than *E. coli.*

***Salmonella* species –** flat blue colonies.

Ps. pyocyanea – green colonies with typical matt surface and rough periphery.

E. faecalis -- Yellow colonies about 0.5mm diameter.

Staph. aureus – deep yellow colonies about 0.75mm diameter, uniform in colour.

Coagulase negative Staphylococci – pale yellow or white, more opaque than *E. faecalis*, often with paler periphery.

Corynebacteria – very small grey colonies.

Lactobacilli – similar to corynebacteria but with a rougher surface.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Proteus mirabilis ATCC ® 10975
Staphylococcus aureus ATCC® 25923

Negative control:
Uninoculated medium

Precautions
Shigella species may not grow on electrolyte-deficient medium.

References

1 Mackey J. P. and Sand G. H. (1966) *B.M.J. 1. 1173.*
2 Sandys G. H. (1960) *J. Med. Lab. Techn. 17. 224.*
3 Mackey J. P. and Sandys G. H. (1965) *B.M.J. 2. 1286–1288.*
4 Guttman D. and Naylor G. R. E. (1967) *B.M.J. 2. 343–345.*

CLED MEDIUM (WITH ANDRADE INDICATOR)

Code: CM423

A modification of the CLED Medium of Mackey and Sandys (B.M.J. 1966, 1, 1173) containing Andrade Indicator to enhance the differentiation of colony characteristics.

Formula	gm/litre
Peptone	4.0
'Lab-Lemco' powder	3.0
Tryptone	4.0
Lactose	10.0
L-cystine	0.128
Bromothymol blue	0.02
Andrade Indicator	0.1
Agar	15.0
pH 7.5 ± 0.2	

Directions
Suspend 36.2g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring.

Description
The formula for this medium is similar to that for CLED Medium, CM301, but with the addition of acid fuchsin which enhances the colonial appearance and aids in identification of the organisms. The colour of the medium differs from that of the standard medium at various pH levels.

Bevis[1] listed the pH and colour as follows:

pH	Colour of Medium
7.4	Deep blue
7.0	Blue grey
6.8	Pale slate grey
6.6	Pinkish grey
6.4	Bright red with slight smokey tint
6.0	Bright red

The medium should not be incubated for longer than 24 hours since, if lactose fermenters predominate, the whole of the medium may turn pink, masking the presence of non-lactose fermenters.

Colonial Characteristics

E. coli – Bright pink semi-translucent colonies with a surrounding pink halo in the medium.

P. mirabilis – Blue-green translucent colonies.

K. aerogenes – Grey-green mucoid colonies.

A. anitratus – Small, grey-green, translucent colonies.

Staph. aureus – Smooth, entire, opaque; bright golden yellow colonies. Lactose fermenting.

Staph. albus – Smooth, entire, opaque porcelain white or very pale pink colonies.

E. faecalis – Similar to *Staph. aureus* but smaller and a much deeper orange yellow colour.

Strep. pyogenes – Small opaque grey-green colonies.

Furniss et al.[2] describe the use of CLED Medium with Andrade's Indicator for rapidly distinguishing vibrios into halophilic and non-halophilic groups. Non-halophilic vibrios grow; halophilic vibrios do not.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Proteus mirabilis ATCC® 10975
Staphylococcus aureus ATCC® 25923

Negative control:
Uninoculated medium.

Precautions
Note the incubation time limit of 24 hours if lactose-fermenting colonies present.

Shigella species may not grow on electrolyte-deficient medium.

References

1 Bevis T. D. (1968) *J. Med. Lab. Technol. 25. 38–41.*
2 Furniss A. L., Lee J. V. and Donovan T. J. (1978) *P.H.L.S. Monograph series, London, H.M.S.O., 11.*

CLOSTRIDIUM DIFFICILE AGAR BASE

Code: CM601

For the isolation of Cl. difficile when used with Selective Supplements SR96 or SR173.

Formula	gm/litre
Proteose peptone	40.0
Disodium hydrogen phosphate	5.0
Potassium dihydrogen phosphate	1.0
Magnesium sulphate	0.1
Sodium chloride	2.0
Fructose	6.0
Agar	15.0
pH 7.4 ± 0.2	

CLOSTRIDIUM DIFFICILE SELECTIVE SUPPLEMENT

Code: SR96

Vial contents (each vial is sufficient for 500ml of medium)

D-cycloserine	125mg
Cefoxitin	4mg

Directions
Suspend 34.5g in 500ml of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Allow to cool to 50°C and add aseptically the contents of 1 vial of Oxoid Clostridium Difficile Supplement SR96 rehydrated with 2ml sterile distilled water, together with 7% (v/v) Defibrinated Horse Blood SR50. Sheep Blood SR51 may be used in place of Horse Blood SR50 but some strains of the organism will show a slightly reduced growth recovery. Mix well and pour into sterile petri dishes.

Description
Clostridium difficile was first isolated in 1935 by Hall and O'Toole[1] who proposed the name 'difficile' because it was very difficult to isolate. In 1940 Snyder[2] isolated *Cl. difficile* from infants aged 10 weeks to 1 year. No further isolations were reported until 1960, when the organism was cultured by McBee[3] from the intestinal contents of a seal, and in 1962 Smith and King[4] reported its presence in human infections.

Toxicogenic isolates of *Cl. difficile* have been demonstrated to be a major cause of antibiotic-associated ileo-caecitis in laboratory animals[5] and pseudomembranous colitis in man[6,7]. Keighley[8] found *Cl. difficile* was associated with colitis and diarrhoea without pseudomembranous changes after antibiotic therapy following gastrointestinal operations.

Hafiz and Oakley[9] devised a medium for the selective isolation of *Cl. difficile* based on the observation that the organism has a high tolerance to cresol, which it produces during its growth, and used Reinforced Clostridial Medium CM151 plus 0.2% phenol or p-cresol.

George et al.[10] in a study of selective media for the routine isolation of *Cl. difficile* from faecal specimens

found this medium was inhibitory compared with growth on blood agar. They recommended the use of a fructose containing nutrient medium plus egg yolk, with D-cycloserine and cefoxitin as selective agents for the isolation of *Cl. difficile.*

The combination of Oxoid Clostridium difficile Agar Base CM601 plus the Selective Supplement SR96 is based on the formulation proposed by George et al.[10]

The selective agents D-cycloserine (500µg/ml) and cefoxitin (16µg/ml) inhibit growth of the majority of Enterobacteriaceae, as well as *Strep. faecalis*, staphylococci, Gram-negative non-sporing anaerobic bacilli and *Clostridia* species. (except *Cl. difficile*) which may be found in large numbers in faecal samples.

Levett[11], noting reports[12,13] that some strains of *Clostridium difficile* had low minimum inhibitory concentrations to both cycloserine and cefoxitin, reduced the antibiotic concentrations to 125µg per ml cycloserine and 4µg per ml cefoxitin and combined this with alcohol shock[14] to compensate for the reduction in selectivity. *Cl. difficile* was isolated from all of the 33 faecal specimens plated on to CCFA Medium containing cycloserine and cefoxitin at 250µg per ml and 8µg per ml respectively, but from only 25/33 specimens plated on to medium containing 500µg per ml cycloserine and 16µg per ml cefoxitin.

The specimen should be treated with alcohol before inoculation (see technique).

It can be expected that medium containing the lower concentration of antibiotics will yield a greater growth of contaminating organisms if antibiotics are used alone, but Levett reported that there was no difference in the growth of contaminating organisms on plates containing either concentration of antibiotics following alcohol shock treatment of the specimen.

Phillips and Rogers[15] have described a simple modification to the medium in which the ability of *Cl. difficile* to produce p-cresol from p-hydroxyphenyl acetic acid is used for the rapid presumptive identification by gas chromatographic detection of the p-cresol.

Addition of 7% horse blood to the agar base increases the recovery of *Cl. difficile* and produces larger colonies compared with Egg Yolk Emulsion used by George et al.[10]

Technique

1 Lightly inoculate the medium with the faecal sample spreading part of the original inoculum in order to obtain well separated colonies.
2 Incubate plates at 35°C for 18–24 hours in a conventional anaerobic gas jar. The use of the Oxoid Anaerobic Jar HP11 with an H_2/CO_2 Gas Generating Kit is strongly recommended. Alternatively use Anaerogen AN025A or AN035A. Anaerogen does not require the addition of water or a catalyst.
3 Colonies of *Cl. difficile* after 48 hours incubation are 4–6mm diameter irregular, raised opaque, grey-white.

Technique for Alcohol Shock Treatment

1 Mix equal parts of industrial methylated spirit or absolute alcohol and the faecal specimen.
2 Homogenise using a vortex mixer.
3 Leave at room temperature for 1 hour.
4 Inoculate on to Clostridium Difficile Selective Agar and incubate anaerobically.

Storage conditions and Shelf life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C no longer than 5–7 days.

Quality Control

Positive control:
Clostridium difficile NCTC 11204

Negative control:
Staphylococcus aureus ATCC® 25923
Escherichia coli ATCC® 25922

Precautions

Colonies of *Cl. difficile* from faecal cultures are smaller when egg yolk is used in place of horse blood.

The Oxoid formula does not contain the neutral red indicator proposed by George et al.[10] because it is designed for use with horse blood. On this medium the typical colour of the colony of *Cl. difficile* will not appear however there will be a fluorescent reaction.

Typical Gram stain morphology of *Cl. difficile* may not be evident in colonies picked from this medium because of the antibiotics present. Subculture to blood agar to obtain characteristic morphology[10].

CLOSTRIDIUM DIFFICILE MOXALACTAM NORFLOXACIN (CDMN) SELECTIVE SUPPLEMENT

Code: CR173

Vial contents (each vial is sufficient for 500ml of medium).

	Mg	Mg/litre
Cysteine hydrochloride	250.0	500.0
Norfloxacin	6.0	12.0
Moxalactam	16.0	32.0

Directions

Aseptically add 2ml of sterile distilled water to a vial and mix gently to dissolve the supplement completely. Avoid frothing. Add to 500ml of Clostridium difficile Agar Base, CM601, prepared as directed and cooled to 50°C. Add 7% v/v of Defibrinated Horse Blood SR50. Mix well and pour into petri dishes.

Description

Cl. difficile CDMN medium is an alternative selective medium based on a formula described by Aspinall *et al*[16] for the isolation of *Cl. difficile* from faeces. It has been found to be significantly more productive than CCFA medium. Inclusion of cysteine hydrochloride speeds the growth rate of *Cl. difficile.* CDMN medium

was reported to isolate 20% more *Cl. difficile* strains than CCFA and the use of norfloxacin and moxalactam as selective agents reduces the number of contaminating micro-organisms by 30% when compared to CCFA[16].

Pre-treatment of specimens with alcohol is not necessary with this medium but its use will further enhance selectivity. See *Cl. difficile* Selective Supplement SR96 for the technique.

Storage conditions and Shelf life
CDMN supplement SR173 should be stored at 2–8°C in the dark.

Quality Control
Positive control:

Clostridium difficile	NCTC 11204

Negative control:

Escherichia coli	ATCC® 25922
Clostridium perfringens	ATCC® 13124

References

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2 Snyder M. L. (1940) *J. Infect. Dis. 66. 1.*
3 McBee R. H. (1960) *J. Bact. 79. 311.*
4 Smith L. D. S. and King E. O. (1962) *J. Bact. 84. 65.*
5 Bartlett J. G., Onderdonk A. B., Cisneros R. L. and Kasper D. L. (1977) *J. Infect. Dis. 136. 701–705.*
6 Bartlett J. G., Chang T. W., Gurwith M., Gorbach S. L. and Onderdonk A. B. (1978) *N. Engl. J. Med. 298. 531–534.*
7 George W. L., Sutter V. L., Goldstein E. C. J., Ludwig S. L. and Finegold S. M. (1978) *Lancet. i. 802–803.*
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10 George W. L., Sutter V. L., Citron D. and Finegold S. M. (1976) *J. Clin. Microbiol. 9. 214–219.*
11 Levett (1985) *J. Clin. Pathol. 38. 233–234.*
12 George W. L., Kirby B. D., Sutter V. L. and Finegold S. M. in Schlessinger D. Editor Microbiology 1979 *Washington D.C. American Society for Microbiology, 2670271.*
13 Dzink J. and Bartlett J. G. (1980) *Antimicrob. Ag. Chemother. 17. 695–698.*
14 Borriello S. P. and Honour H. (1981) *J. Clin. Pathol. 34. 1124–1127.*
15 Philips K. D. and Rogers P. A. (1981) *J. Clin. Pathol. 34. 643–644.*
16 Aspinall S.T. and Hutchinson D.N. (1992) *J. Clin. Pathol. 45. 812–814.*

COLUMBIA BLOOD AGAR BASE

Code: CM331

A multi-purpose medium suitable for the cultivation of fastidious organisms.

Formula	gm/litre
Special peptone	23.0
Starch	1.0
Sodium chloride	5.0
Agar	10.0
pH 7.3 ± 0.2	

Directions
Add 39g to 1 litre of distilled water. Boil to dissolve the medium completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add 5% sterile defibrinated blood.

Description
Traditionally, blood agar bases have been either casein hydrolysate or meat infusion media. The advantage of the first lies in the rapid production of large colonies, and of the second in clearly defined zones of haemolysis and good colonial differentiation.

Columbia Agar Base (Ellner et al.[1]) combines the virtues of both these types of media to give an improved all-round performance.

This new base has shown versatility and superior performance in several applications.

BRUCELLA

To prepare a selective medium add Brucella Selective Supplement SR83 to 500ml of sterile, molten Columbia Blood Agar Base, containing 5–10% v/v inactivated horse serum and 1% w/v dextrose[2,3].

CAMPYLOBACTER AND HELICOBACTER

To prepare a selective medium add:

Campylobacter Supplement (Skirrow) SR69 4
or Campylobacter Supplement (Butzler) SR85[5,6]
or Campylobacter Supplement (Blaser-Wang) SR98[7,8,9]
or Helicobacter pylori Supplement SR147 to 500ml of sterile, molten Columbia Agar Base containing Campylobacter Growth Supplement SR84[10,11] and 5–7% v/v horse or sheep blood.

Egg Yolk Emulsion Agar made using Oxoid Columbia Agar Base and Egg Yolk Emulsion SR47 has been shown to be a satisfactory isolation medium for *Helicobacter pylori*[12].

GRAM-POSITIVE COCCI

see Staph/Strep Selective Medium on page 2-194.
see Streptococcus Selective Medium on page 2-195.

GARDNERELLA

see Gardnerella vaginalis Selective Medium on page 2-99.

OTHER APPLICATIONS

Elek Test
Columbia Agar Base with added sterile serum provides an efficient *Corynebacterium diphtheriae* virulence test medium. After following the established technique, lines of toxin-antitoxin precipitation are clearly visible in 48 hours.

Nagler Test
The addition of 5ml Fildes Extract SR46 and 10ml of Egg Yolk Emulsion SR47 to 100ml of sterile, molten Columbia Blood Agar Base will provide a diagnostic medium for *Clostridium perfringens*, when used with *C. perfringens* antitoxin (Nagler reaction) and neomycin (100–125m/ml)[13].

Reverse Camp Test for Clostridium perfringens
The reverse CAMP test[14] is a highly sensitive and specific test for *C. perfringens* which may be used as an alternative to the Nagler test.

Technique
Inoculate the culture suspected to be *C. perfringens* in a straight line across a plate of Columbia sheep blood

agar. Streak an overnight (or older) culture of *Streptococcus agalactiae* known to produce the CAMP factor at right angles to the first inoculation taking care that the lines do not touch. Incubate anaerobically at 35–37°C for 18–24 hours.

A positive reverse CAMP test is indicated by the formation of an ''arrowhead'' of haemolysis between the lines of the *C. perfringens* and *Strep. agalactiae* growth.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium at 2–8°C.

Quality Control
Columbia Agar
Positive control:
Staph. aureus ATCC® 25923
Strept. pyogenes ATCC® 19615

Negative control:
Uninoculated plate

Brucella Medium
Positive control:
**Brucella abortus* ATCC® 4315

Negative control:
Esch. coli ATCC® 25922

Campylobacter Media
Positive control:
C. jejuni ATCC® 29428

Negative control:
Esch. coli ATCC® 25922

Staph Medium Strep
Positive control:
Staph. aureus ATCC® 25923
Strept. pyogenes ATCC® 19615

Negative control:
Esch. coli ATCC® 25922

Streptococcus Selective Medium
Positive control:
Strept. pyogenes ATCC® 19615

Negative control:
Staph. aureus ATCC® 25923

Gardnerella Selective Medium
Positive control:
G. vaginalis ATCC® 14018

Negative control:
Bact. fragilis ATCC® 25285

Precautions
**Brucella* cultures are highly infective and must be handled under properly protected conditions. Incubate in 5–10% carbon dioxide atmosphere for 24–48 hours.

Campylobacter species are best grown at 42°C (except *C. fetus* subsp. *fetus*) in a micro-aerophilic atmosphere (Oxoid Campylobacter Gas Generating Kit BR56 or BR60 or CampyGen CN025/CN035).

Staph./Strep. supplemented plates should be incubated aerobically at 35°C for 18 hours. Incubation in carbon dioxide-enriched air will cause inhibition of staphylococcal growth[15].

Strep. supplemented plates may be incubated aerobically or anaerobically at 35°C for 18 hours.

Prepared plates of both supplemented media should be used within 18 hours of preparation for maximum selectivity. Gardnerella supplemented plates should be incubated at 35°C for 48 hours in an atmosphere containing 7% carbon dioxide.

Carry out confirmatory tests on all colonies from horse blood medium and on beta-haemolytic colonies from human or rabbit blood medium.

Incubate plates of Clostridium E-Y Agar anaerobically at 35°C for 18 hours, look for lecithinase activity (pearly layer) and for proteolysis. Lecithinase activity is inhibited in the presence of specific anti-toxin.

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COOKED MEAT MEDIUM

Code: CM81

An excellent medium for the primary growth and maintenance of aerobic and anaerobic organisms.

Formula	gm/litre
Heart muscle	454.0
Peptone	10.0
'Lab-Lemco' powder	10.0
Sodium chloride	5.0
Glucose	2.0
pH 7.2 ± 0.2	

Directions
Suspend 10g in 100ml of distilled water (or 1g amounts in 10ml volumes of water in tubes). Allow to stand for 15 minutes until the meat particles are thoroughly wetted. Sterilise by autoclaving at 121°C for 15 minutes. Do not cool the bottles rapidly because ebullition will expel the meat particles from the containers.

Description
Cooked Meat Medium prepared from heart tissue is a well established medium for the cultivation of anaerobic and aerobic organisms[1].

It has the ability to initiate growth of bacteria from very small inocula and to maintain the viability of cultures over long periods of time. Mixed cultures of bacteria survive in Cooked Meat Medium without displacing the slower growing organisms. The products of growth do not rapidly destroy the inoculated organisms and therefore it is an excellent medium for the storage of aerobic and anaerobic bacteria.

The addition of glucose in the formulation allows rapid, heavy growth of anaerobic bacteria in a short time and leads to a more rapid identification of important anaerobes. The improved growth also enhances GLC identification of anaerobic bacteria.

The improved clarity of the supernatant broth permits earlier detection of growth especially when combined with the increased growth of most organisms. Slower growing isolates will yield detectable growth in 45 hours incubation.

Technique
Anaerobic Culture. It is preferable to use freshly reconstituted and sterile medium which is inoculated as soon as it has cooled to approximately 35°C. Tubes which are not used on the day of preparation should be placed in a boiling water bath or steamer for about 15 minutes to remove dissolved oxygen. They should be allowed to cool without agitation and then inoculated.

Inoculation should be made near the bottom of the tube in the meat particles.

Clostridia may be divided into two main groups by their action on the medium.

(i) Saccharolytic Organisms
There is rapid production of acid and gas but no digestion of the meat. Cultures may have a slightly sour smell, with reddened protein.

(ii) Proteolytic Organisms
Proteolysis causes decomposition of the meat with the formation of foul-smelling sulphur compounds and blackening. However, some saccharolytic strains also produce H_2S which will cause blackening but to a lesser degree.

Aerobic Culture
The tube of medium is incubated with the cap loose and no seal is required. Aerobes grow at the top whilst more anaerobic species grow deeper in the medium.

Incubation
Aerobic organisms: incubate up to 7 days at 35°C with loosened caps, examine daily for turbidity, gas or changes in the meat particles.

Anaerobic organisms: use freshly reduced medium and incubate up to 21 days at 35°C, examine daily for changes in the medium; make films and subculture at intervals.

Maintenance of stock cultures: hold at room temperature after the initial incubation at 35°C. Subculture every 4–6 months.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at room temperature, in the dark with tightened caps, up to 6 months.

Quality Control
Positive proteolysis:
Clostridium histolyticum ATCC® 19401

Positive saccharolysis:
Clostridium perfringens ATCC® 13124

Negative control:
Uninoculated medium

Precautions
The excellent recovery properties of Cooked Meat Medium mean that mixed cultures commonly result from sample inoculation.

Blackening of the medium will not take place if the pH is acid.

Carbohydrate fermentation may inhibit proteolysis.

Reference
1 Robertson M. (1916) *J. Path. Bact. 20. 327–349.*

CORN MEAL AGAR

Code: CM103

A recommended medium for chlamydospore production by Candida albicans and for the maintenance of fungal stock cultures.

Formula	gm/litre
Corn Meal Extract (from 50 grams whole maize)	2.0
Agar	15.0
pH 6.0 ± 0.2	

Directions
Suspend 17g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Corn Meal Agar is a well established mycological medium which is a suitable substrate for chlamydospore production by *Candida albicans* and the maintenance of fungal stock cultures.

When grown on this medium, microscopic examination of *Candida albicans* shows the characteristic chlamydospore production which is an

accepted criterion for the identification of this species. Prospero and Reyes[1] investigated the use of corn meal agar, soil extract agar, and purified polysaccharide medium for the morphological identification of *C. albicans*. Out of 290 yeast colonies isolated on Sabouraud agar, corn meal agar stimulated the production of chlamydospores in 149 colonies (51%), soil extract agar in 103 (36%) and purified polysaccharide medium in 94 (32%).

The addition of 'Tween 80' (e.g. 1%) to Corn Meal Agar greatly enhances the development of chlamydospores on the medium[2,3,4,5,6].

Mackenzie[7] found that all 163 isolates of *Candida albicans* obtained from laboratories in the United Kingdom produced chlamydospores on Oxoid Corn Meal Agar but Dawson[8] using only 27 isolates of *Candida albicans*, found that Oxoid Czapek Dox Agar and rice infusion agar were slightly superior for chlamydospore production.

Corn meal agar is a nutritionally impoverished medium and so may be employed for the maintenance of stock cultures of fungi, especially the black-pigmented varieties.

The addition of glucose (0.2g% w/v) to Corn Meal Agar will enhance the chromogenesis of some species of *Trichophyton* e.g. *T. rubrum*[9].

Technique
A single petri dish containing Corn Meal Agar may be used to identify four or five different colonies of *Candida* grown on Sabouraud Dextrose Agar CM41. Using a straight wire, pick a colony off the surface of the latter medium and make a deep cut in the Corn Meal Agar (i.e. a horizontal furrow). Repeat for each colony. Place a flamed sterile coverslip over the line of inoculum. After incubation for 24 to 48 hours at 22°C, the streaks are examined microscopically, through the cover slip, using a low power objective. Along such streaks, *C. albicans* produces mycelium-bearing ball-like clusters of budding cells and the characteristic thick-walled round chlamydospores[9].

The addition of 0.001g% w/v Trypan blue to Corn Meal Agar provides a contrasting background for the observation of characteristic morphological features of yeast cultures[10].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium at 2–8°C.

Quality Control
Chlamydospore Production
Positive control:
Candida albicans ATCC® 10231

Negative control:
Candida krusei ATCC® 6258

Precautions
Glucose supplemented Corn Meal Agar should not be used for chlamydospore production.

Corn Meal Agar with 'Tween 80' (or other wetting agents) will allow *C. stellatoides* and *C. tropicalis* to produce chlamydospores.

Some *Candida* strains lose their ability to produce chlamydospores after repeated subculturing.

References

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CROSSLEY MILK MEDIUM

Code: CM213

This medium is suitable for use where Litmus Milk was previously specified.

Formula	gm/litre
Skim milk powder	100.0
Peptone	10.0
Bromocresol purple	0.1
pH 6.8 ± 0.2	

Directions
Cream 110g of the powder with a little distilled water and gradually dilute to 1 litre with continuous mixing. Tube in 10ml quantities and autoclave at 121°C for 5 minutes.

Description
A simple medium originally described by Crossley[1] for the routine examination of canned food samples for anaerobic bacteria.

This medium was evolved as the result of comparative trials carried out by Crossley with several standard media. It is capable of giving rapid growth without the use of special anaerobic apparatus, yet the bacteria detected may be provisionally identified by their reactions upon the medium.

Crossley milk medium is recommended, in the second edition of Tanner's 'The Microbiology of Foods'[2], for the examination of meat, meat products, and canned foods for sporing anaerobes.

Technique
The following method of examination is suggested: Inoculate 10ml of Oxoid Crossley Milk Medium with 1.5 to 2.0g of the sample. Incubate for 3 to 4 days at 37°C and examine for the following striking and characteristic reactions.

	Reaction	Organism Indicated
1	Neutral or alkaline pH (purple colour), gas production, soft curd followed by rapid digestion of casein, often to a clear brown liquid, formation of black sediment accompanied by typical foul odour.	*Cl. putrificum* *Cl. sporogenes* *Cl. flabelliferum* *Cl. oedematiens* *Cl. histolyticum*
2	No initial change of pH, formation of soft curd within 2–3 days, slight gas formation. Complete digestion later with alkaline reaction, no odour.	*Cl. centrosporogenes*
3	Slight acidity (pale yellow colour), formation of soft curd, and whey. Slight gas production.	*Cl. sphenoides*
4	Acid (bright yellow colour), formation of firm clot and gas. Bleaching of the indicator may sometimes occur.	*Cl. butyricum*
5	(a) Acid, formation of 'stormy' clot. (b) Acid and 'stormy' clot but with less gas and cloudy whey.	(a) *Cl. welchii* (*Cl. perfringens*) (b) Usually *Cl. tertium*
6	Strong alkaline pH, with peptonisation commencing at the surface and spreading downwards. Digestion not complete, no blackening, no odour, no gas production.	*B. subtilis, B. vulgatus*
7	Acid and clot, or slightly acid only. Peptonisation in some cases.	*B. cereus, B. coagulans, B. silvaticus* and various cocci (More detailed tests required)

Crossley[1] modified his medium, by the addition of 20% (w/v) of autoclaved meat or fish paste, so that it was suitable for the examination of vegetable and dairy products.

The medium recommended by Jepsen[3] in 'Meat Hygiene' published by the World Health Organization, is Crossley Milk Medium modified by the addition of 20% (w/v) of cooked fish; it is suitable for the examination of meat products for clostridia, and gives diagnostic reactions essentially similar to those outlined above. Riemann[4] modified Crossley Milk Medium by the addition of 0.08% of cysteine hydrochloride, before autoclaving, or by the addition of 1ml of a sterile 10% sodium thioglycollate solution just before use.

Any of the above additions may be used to supplement Oxoid Crossley Milk Medium.

References

1 Crossley E.L. (1941) *J. Soc. Chem. Ind. 60, 131–136.*
2 Tanner F.W. (1944) *'The Microbiology of Foods' 2nd ed., Garrard Press, London, pp. 893, 1001–1002.*
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CZAPEK DOX AGAR (MODIFIED)

Code: CM97

A solid defined medium for the cultivation of those fungi and bacteria which are able to utilise sodium nitrate as the sole source of nitrogen. The acidity of the medium may be increased for the cultivation of acidophilic organisms such as yeasts.

Formula	gm/litre
Sodium nitrate	2.0
Potassium chloride	0.5
Magnesium glycerophosphate	0.5
Ferrous sulphate	0.01
Potassium sulphate	0.35
Sucrose	30.0
Agar	12.0
pH 6.8 ± 0.2	

Directions
Suspend 45.4g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring. If it is required to adjust the reaction to pH 3.5 ± add 10ml of Lactic Acid 10% SR21 per litre after sterilisation.

Description
Czapek Dox Agar (Modified) is a medium containing sodium nitrate as the sole source of nitrogen, it is one of the most useful solid media for the general cultivation of fungi.

In the Oxoid medium magnesium glycerophosphate and potassium sulphate replace the magnesium sulphate and potassium phosphate of the original. This modification prevents the precipitation of magnesium phosphate. The medium is also a highly satisfactory substrate for chlamydospore production by *Candida albicans*[1].

Dawson[1] employed Oxoid Czapek Dox Agar (Modified) in her technique for the identification of *Candida albicans* by chlamydospore formation in primary culture, using swabs taken from the mouth and from the vagina. Identification was usually possible within 24 hours. The Oxoid medium showed good chlamydospore production whereas the original formulation did not. After 24 hours incubation 23 out of 27 *C. albicans* strains had formed chlamydospores on Oxoid Czapek Dox Agar (Modified), 21 on rice infusion agar, 10 on Oxoid Corn Meal Agar and 10 on a corn meal agar made in the laboratory. After 48 hours 25 strains had formed chlamydospores on both the Oxoid medium and the rice agar, 24 on Oxoid Corn Meal Agar and 20 on the laboratory medium. Dawson concluded that the Oxoid Czapek Dox medium and the rice infusion agar were the most satisfactory media. None of 14 strains of unidentified yeasts formed chlamydospores on any medium.

Smith[2] cited the following recommendations for the use of Czapek Dox Agar for taxonomic studies: by Thom and Church[3] for *Aspergillus*; by Thom[4] and by Raper and Thom[5] for *Penicillium*; and by Wakesman[6] for actinomycetes.

Technique
General Cultivation
To avoid excessive condensation cool the molten medium to 50°C before pouring approximately 12ml into each 9cm diameter petri dish. Store the poured plates in an inverted position and inoculate using needle or wire, with the plate still inverted in order to avoid scattering stray fungal spores over the surface of the medium. Time and temperature of incubation vary considerably according to the species being cultivated, as a general guide, incubate for 1–2 weeks at 25°C. Most *Penicillium* species have an optimum growth temperature between 20° and 25°C, whilst many *Aspergillus* species grow best at about 30°C. However, different fungi grow over a wide range of temperatures; *Aspergillus fumigatus* grows well at 50°C (Smith[2]) and *Cladosporium herbarum* will grow on meat at –6°C[7,8].

Identification of *Candida albicans*[1].
1 Using an inoculating needle (previously flamed, cooled and rubbed against the swab) cut across and through the medium in a Czapek Dox Agar plate to the base of the petri dish. With the same needle, raise the medium along the whole of one side of the cut – so that the inoculum is spread between the agar and the base of the dish.
2 Incubate the inoculated plates for 24 hours at 28°C.
3 Using a low-power objective, microscopically examine the unopened plates for chlamydospores through the base of each dish. Alternatively, remove the tops of the dishes, and examine through the top of the medium.
4 If no chlamydospores are seen, incubate for a further 24 hours and re-examine.

CZAPEK DOX LIQUID MEDIUM (MODIFIED)

Code: CM95

A defined fluid medium for the cultivation of those fungi and bacteria which are able to utilise sodium nitrate as the sole source of nitrogen.

Formula	gm/litre
Sodium nitrate	2.0
Potassium chloride	0.5
Magnesium glycerophosphate	0.5
Ferrous sulphate	0.01
Potassium sulphate	0.35
Sucrose	30.0
pH 6.8 ± 0.2	

Directions
Add 33.4g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A defined fluid medium for the cultivation of fungi and bacteria capable of utilising sodium nitrate as the sole source of nitrogen.

Storage conditions and Shelf life
Store dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared agar plates at 2–8°C.
Store tubes of broth at 15–25°C.

Quality Control
Positive control:
Aspergillus niger ATCC® 9642
Candida albicans ATCC® 10231

Negative control:
Uninoculated medium

References

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6 Wakesman S. A. (1931) *'Principles of soil Microbiology' Bailliere Tindall and Cox, London.*
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DCLS AGAR

Code: CM393

A modified DCA containing sucrose to improve the accuracy of recognition of pathogenic enterobacteriaceae.

Formula	gm/litre
Special peptone	10.0
Sodium citrate	10.5
Sodium thiosulphate	5.0
Lactose	5.0
Sucrose	5.0
Sodium desoxycholate	2.5
Neutral red	0.03
Agar	12.0
pH 7.2 ± 0.2	

Directions
Suspend 50g in 1 litre of distilled water. Bring to the boil to dissolve the medium completely. Cool to 50°C and pour plates. DO NOT AUTOCLAVE.

Description
DCLS Agar is a modified form of Desoxycholate Citrate Agar[1] which includes sucrose in its formulation. The addition of this fermentable carbohydrate increases the usefulness of the medium because non-pathogenic sucrose-fermenting organisms may be recognised by their red colonies, e.g. some *Proteus, Enterobacter* and *Klebsiella* species.

DCLS Agar reduces the number of false-positive sub-cultures when picking colonies and therefore improves the efficiency of isolation.

The Special peptone, used in DCLS Agar, includes the nucleic acid factors, vitamins and carbon compounds of meat extract, as well as a rich variety of polypeptides. It has improved the growth of shigellae and salmonellae, but it should be noted that *Sh. sonnei* may exhibit a translucent, pink colony which should not be confused with the red *Esch. coli* colony.

The selectivity of DCLS Agar is similar to Desoxycholate Citrate Agar and it will grow *Vibrio* species, as well as salmonellae and shigellae, whilst inhibiting the growth of *Esch. coli.*

DCLS Agar may be inoculated directly from the specimen, or inoculated after enrichment through Selenite Broth CM395 and L121, Muller-Kauffmann Tetrathionate Broth CM343 or Tetrathionate Broth CM29. The plates should be incubated overnight (18–24 hours) at 35°C and examined for the presence of pale, translucent or colourless colonies. Sub-cultures can be made into confirmatory media such as Kligler Iron Agar CM33 or Triple Sugar Iron Agar CM277 or picked for transfer to nutrient broth for subsequent motility tests and serological agglutinations.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared agar plates at 2–8°C.

Quality Control
Positive control:
Lactose/sucrose fermenters
Proteus vulgaris ATCC® 13315
Non-lactose/sucrose fermenters
Salmonella typhimurium ATCC® 14028

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions
Boil the medium for the minimal period of time to get the agar into solution. Overheating reduces the agar gel strength and increases the degree of inhibition. It is therefore important not to hold the molten medium at 50°C for more than the short time required to distribute it into dishes.

Reference
1 Leifson E. (1935) *J. Path. Bact. 40. 581–599.*

DERMASEL AGAR BASE

Code: CM539

A selective medium for dermatophyte fungi recommended for the examination of hair, skin scrapings, nails, etc.

Formula	gm/litre
Mycological peptone	10.0
Glucose	20.0
Agar	14.5

DERMASEL SELECTIVE SUPPLEMENT

Code: SR75

Vial contents (each vial is sufficient for 500ml of medium)

Cycloheximide	200mg
Chloramphenicol	25mg

Directions
Suspend 44.5g in 1 litre of distilled water and heat gently to dissolve completely. Add the contents of 1 vial of Antibiotic Supplement SR75, reconstituted with 3mls of ethanol, to each 500ml of medium to give a level of cycloheximide 0.4g/l and chloramphenicol 0.05g/l. Mix gently and sterilise by autoclaving at 121°C for 10 minutes. Avoid overheating at any time.

Description
Oxoid Dermasel Agar CM539 is used for the primary isolation and identification of dermatophyte fungi from hair, nails or skin scrapings.

Emmons[1] suggested that media for growth of dermatophytes should have a pH of 6.8–7.0 rather than pH 5.6 as is often recommended. A near neutral pH is better for the growth of some fungi and the acid pH used to suppress bacterial contaminants can be replaced by antibiotics.

The addition to the medium of Oxoid Antibiotic Supplement SR75 to give a level of cycloheximide 0.4g/l and chloramphenicol 0.05g/l renders the medium selective for dermatophytes, inhibiting the growth of saprophytic fungi, yeasts and bacterial skin flora[2].

The chloramphenicol and cycloheximide supplement SR75 reduces the potential risk to health from these antibiotics. To include them in the powder mix could allow them to be scattered as dust whilst weighing the medium. It also ensures a fixed, accurate dose of antibiotic that has been protected from degradation on storage.

The addition of cycloheximide and an anti-bacterial agent has been reported to improve considerably the isolation of dermatophytes, especially when the inoculum, such as horse hair was heavily contaminated[3,4]. The presence of staphylococci, which may grow in the absence of the antibiotic has been shown to prevent the *in-vitro* growth of *Trichophyton rubrum*[5].

The presence of cycloheximide in the medium inhibits the growth of *Trichosporon cutaneum*, *Candida parasilosis*, *Candida krusei*, *Aspergillus*, *Penicillium*, *Fusarium* and *Cephalosporium* species which have been associated with diseased nails[6,7].

The incorporation of griseofulvin at a level of 20µg/ml into one of paired tubes of selective media has been recommended as an additional aid in the diagnosis of dermatophytosis[8]. The absence of growth, on the medium containing griseofulvin provides presumptive identification of a dermatophyte fungus.

Dermatophyte fungi cultured on Oxoid Dermasel Agar show characteristic colonial morphology with

typical pigmentation. Macroconidia and microconidia are typical for the species when studied microscopically.

Technique
Oxoid Dermasel Agar CM539 may be prepared as slopes in test tubes with loose caps to ensure adequate aeration, or in vented petri dishes.

Small, pin head sized samples of the test material are stabbed into the surface of the agar. A number of samples may be inoculated on to the same surface.

The medium is incubated at 22°C to 30°C and examined at regular intervals for two to four weeks.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared agar plates at 2–8°C.

Quality Control
Positive control:
Trichophyton rubrum ATCC® 28191
Candida albicans ATCC® 10231

Negative control:
Aspergillus niger ATCC® 9642
Escherichia coli ATCC® 25922

Precautions
This medium should not be used if agents causing systemic mycoses are being sought[9]. If such agents are suspected e.g. cryptococcus, histoplasma etc., either Dermasel Agar Base CM539 *without antibiotic supplement* must be used in parallel or Brain Heart Infusion Agar CM375.

If the fungal agent sought is suspected to be nutritionally fastidious, the use of Brain Heart Infusion Agar is particularly helpful.

Supplement SR75 contains a toxic concentration of cycloheximide. Note the precautions to be taken under HAZARDS page 2–7.

References

1 Emmons C. W., Binford C. H. and Utz J. P. (1963) *Medical Mycology. Henry Kimpton.*
2 Georg L. K., Ajello L. and Papageorge C. (1954) *J. Lab. Clin. Med. 44. 422.*
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4 Merz W. G., Berger C. L. and Silva-Huntar M. (1970) *Arch. Derm. 102. 545–547.*
5 Silva M., Kesten B. M. and Benham R. W. (1955) *J. Invest. Derm. 25. 311–328.*
6 Zaias N. (1966) *Sabouraudia 5. 99–103.*
7 Rosenthal S. A., Stritzler R. and Villafane J. (1968) *Arch. Derm. 97. 685–687.*
8 Blank H. and Rewbell G. (1965) *Arch. Derm. 92. 319–322.*
9 McDonough E. S., Georg L. K., Ajello L. and Brinkman S. (1960) *Mycopath et Mycol. Appl. 13. 113–115.*

DESOXYCHOLATE AGAR

Code: CM163

A differential medium for the enumeration of coliforms in dairy products. It may be employed as a non-selective medium for the isolation of enteric pathogens.

Formula	gm/litre
Peptone	10.0
Lactose	10.0
Sodium desoxycholate	1.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	2.0
Ferric citrate	1.0
Sodium citrate	1.0
Neutral red	0.03
Agar	15.0
pH 7.1 ± 0.2	

Directions
Suspend 45g in 1 litre of distilled water. Bring to the boil over gauze and flame to dissolve the medium completely. Agitate to prevent charring.

THIS MEDIUM IS HEAT SENSITIVE. AVOID EXCESSIVE OR PROLONGED HEATING DURING RECONSTITUTION. DO NOT AUTOCLAVE OR REMELT.

Description
Desoxycholate Agar is a differential medium for the direct count of coliforms in dairy products (American Public Health Association[1]). It may also be employed for the isolation of enteric pathogens from rectal swabs, faeces, or other specimens.

The medium may be used in a 'pour-plate' technique or as a surface inoculated medium. A thin layer of uninoculated desoxycholate agar poured over the surface of a gelled 'pour-plate' assists subsequent counting.

Technique

Enumeration of Coliforms in Milk and Cream (APHA[1])

1 Pipette 1–4ml of the sample (or decimal dilution of the sample) into a sterile petri dish.
2 Cool freshly prepared Desoxycholate Agar to 42–44°C and add 10–20ml to each dish.
3 Mix the contents of the dishes by gentle tilting and rotation.
4 Allow the plates to solidify and pour on an overlay of 3–4ml of uninoculated Desoxycholate Agar.
5 When the overlay has set, invert the plates and incubate them for 18–24 hours at 35°C.
6 Count all dark red colonies measuring at least 0.5mm in diameter, and calculate the number of coliform colonies per millilitre or gram of original sample.

Isolation of Enterobacteriaceae
It is advisable to use Desoxycholate Agar in parallel with other plating media for this purpose.

Lightly inoculate a Desoxycholate Agar plate with faeces, rectal swab, or enrichment culture. Incubate for 18–24 hours at 35°C and examine. Non-lactose

fermenters of enteric origin form colourless colonies. Non-lactose fermenters which are not of enteric origin are generally inhibited by the sodium desoxycholate in the medium. Identify suspect colonies in the usual manner.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared agar plates at 2–8°C.

Quality Control
Positive control:
Lactose Fermenters
Escherichia coli ATCC® 25922
Klebsiella oxytoca NCTC 8167

Non-Lactose Fermenters
Shigella sonnei ATCC® 25931

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions
As with all desoxycholate media, this medium is heat sensitive. Observe the precautions stated under Directions.

Reference
1 American Public Health Association (1978) *'Standard Methods for the Examination of Dairy Products' 14th ed,. APHA Inc., New York, pp. 58–59.*

DESOXYCHOLATE CITRATE AGAR

Code: CM35

A modification of Leifson's medium for the isolation of intestinal pathogens.

Formula	gm/litre
'Lab-Lemco' powder	5.0
Peptone	5.0
Lactose	10.0
Sodium citrate	5.0
Sodium thiosulphate	5.0
Ferric citrate	1.0
Sodium desoxycholate	2.5
Neutral red	0.025
Agar	15.0
pH 7.0 ± 0.2	

Directions
Suspend 48.5g in 1 litre of distilled water. With frequent agitation bring to the boil over a gauze and flame to dissolve completely. Mix well and pour plates immediately. Dry the agar surface before use.

THIS MEDIUM IS HEAT SENSITIVE. AVOID EXCESSIVE OR PROLONGED HEATING DURING RECONSTITUTION. DO NOT AUTOCLAVE, OR REMELT.

Description
An Oxoid modification of Leifson medium[1], for the isolation and maximum recovery of intestinal pathogens. It is less selective and inhibiting than Desoxycholate Citrate Agar (Hynes) but colonial characteristics are identical on the two media.

See Desoxycholate Citrate Agar (Hynes) CM227 for the description of colonies but note that DCA CM35 provides an opaque background against which one may more easily discern the clearing produced by alkali-producing pathogens.

The use of a less selective medium for direct sampling of faeces and a more selective medium for post-enrichment sampling, would be advantageous. Similarly, the less inhibitory medium is often preferable when shigellae are being sought as well as salmonellae[2].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared agar plates at 2–8°C.

Quality Control
Positive controls:
Salmonella typhimurium ATCC® 14028
Shigella sonnei ATCC® 25931

Negative control:
Enterococcus faecalis ATCC® 29212

Precautions
Observe the precautions about overheating shown under Directions.

The medium is best used freshly prepared.
Stock cultures of *Shigella* species may become predominantly in the R-phase when subcultured away from DCA media. Such cultures are difficult to use for control purposes without first heavily streaking the cultures on DCA plates and picking off the few S-phase colonies i.e. the macro-colonies on the agar surface, for further subculture.

When making biochemical tests on colonies picked from the surface of DCA plates, purity subcultures should be carried out because the colony may be contaminated with *Escherichia coli* present as micro-colonies.

References
1 Leifson E. (1935) *J. Path. Bact. 40. 581–599.*
2 Fricker C.R. (1987) *J. Appl. Bact. 63. 99–116.*

DESOXYCHOLATE CITRATE AGAR (HYNES)

Code: CM227

A selective medium for the isolation of Salmonella and Shigella organisms.

Formula	gm/litre
'Lab-Lemco' Powder	5.0
Peptone	5.0
Lactose	10.0
Sodium citrate	8.5
Sodium thiosulphate	5.4
Ferric ammonium citrate	1.0
Sodium desoxycholate	5.0
Neutral red	0.02
Agar	12.0
pH 7.3 ± 0.2	

Directions
Suspend 52g in 1 litre of distilled water. Bring to the boil over gauze and flame, to dissolve completely. Agitate to prevent charring. Dry the agar surface before use.

THIS MEDIUM IS HEAT SENSITIVE: AVOID EXCESSIVE OR PROLONGED HEATING DURING RECONSTITUTION. DO NOT AUTOCLAVE OR REMELT.

Description
An improved medium, based on the Hynes[1] modification of Leifson medium for the isolation of salmonellae and shigellae.

The improvement gives larger and more numerous colonies of *Shigella* species which can easily be picked off and emulsified in saline for slide agglutination tests.

Desoxycholate Citrate Agar (Hynes) is more selective than CM35. In particular, CM227 is more inhibitory to coliforms and *Proteus* species.

Technique
Inoculate the medium heavily with faeces or rectal swabs, spreading part of the original inoculum in order to obtain well separated colonies on some portion of the plate. Incubate for 18–24 hours at 35°C. If organisms are late developers or if no non-lactose fermenters are observed, incubate for a further 24 hours.

Colonies may be picked directly off the medium for serological and biochemical tests. It should be noted that *Escherichia coli* survives on the medium even though it does not usually grow – therefore colonial purity should be established by subculture on to a differential but less inhibitory medium, e.g. MacConkey Agar CM7.

Colonial Characteristics
(Following incubation at 35°C.)
The medium is clear and pale pink. Lactose fermenting organisms produce pink colonies and may be surrounded by a zone of precipitated desoxycholic acid, which is due to acid production. The colonies of non-lactose fermenters are colourless, and due to their alkaline reaction they are surrounded by a clear orange-yellow zone of medium.

Escherichia coli -- Most strains are inhibited, but the few strains which grow produce pink umbilicated colonies 1–2mm in diameter which may be surrounded by a zone of precipitation. *Aerogenes* colonies are domed and mucoid.

Shigella sonnei -- the colonies grow from 1mm diameter after 18 hours incubation to 2mm after 38 hours; they are smooth and initially colourless, becoming pale pink on further incubation due to late lactose fermentation.

Shigella flexneri -- colonies are colourless and similar in appearance to those of *Shig. sonnei*, but often with a narrow plane periphery round a central dome.

Salmonella paratyphi B – from 1mm diameter after 18 hours incubation to 2–4mm on the second day, when they are slightly opaque, dome-shaped, with a central black dot.

Salmonella typhosa – 0.25 to 1mm in diameter after 18 hours and pale pink, a day later they are flat, conical, 2mm in diameter, colourless and slightly opaque, often with a central grey dot.

Other Salmonella colonies -- similar to those of *Salm. paratyphi B*. Non-pathogenic non-lactose fermenters, such as *Proteus* and *Pseudomonas* species, grow on the medium and may produce colonies which closely simulate those of the salmonellae or shigellae. *Proteus* colonies are often glossy (more translucent than those of the pathogens), with a large central black dot and a 'fishy' odour.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared agar plates at 2–8°C.

Quality Control
Positive control:
Salmonella typhimurium ATCC® 14028
Shigella sonnei ATCC® 25931

Negative control:
Enterococcus faecalis ATCC® 29212

Precautions
Note the precautions listed under Desoxycholate Citrate Agar CM35.

Reference
1 Hynes M. (1942) *J. Path. Bact. 54. 193–207.*

DEXTROSE TRYPTONE AGAR

Code: CM75

For the detection and enumeration of 'flat-sour' thermophiles and mesophiles in food products. Acid producing organisms such as 'flat-sour' thermophiles form yellow colonies surrounded by a yellow zone.

Formula	gm/litre
Tryptone	10.0
Dextrose	5.0
Bromocresol purple	0.04
Agar	12.0
pH 6.9 ± 0.2	

Directions
Suspend 27g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A bacteriologically controlled medium for the detection and enumeration of thermophilic and mesophilic organisms in food products, etc.

Dextrose Tryptone Agar, evolved as the result of several years research by Williams[1] is most suitable for the cultivation and enumeration of the thermophilic bacteria causing 'flat-sour' spoilage of canned food. Its use for routine cultural purposes is recommended by Cameron[2] and the Association of

Official Analytical Chemists[3]. Dextrose Tryptone Agar is also recommended by:

1 Tanner[4] for the examination of canned food, sugar, and starch for thermophilic bacteria of the *Bacillus stearothermophilus* type (i.e. 'flat-sour' spoilage bacteria).
2 The American Public Health Association[5] for the enumeration of mesophilic and thermophilic aerobic bacteria in sweetening agents used in frozen dairy foods.
3 The National Canners Association[6] for determination of the total plate and 'flat-sour' count of thermophilic bacteria spores in ingredients, such as sugar and starch.
4 The American Public Health Association[7] for the enumeration of mesophilic organisms and 'flat-sour' spores in sugars, starches and other complex carbohydrates; and for the enumeration of 'flat-sour' thermophiles in cereals and cereal products, dehydrated fruits and vegetables, and spices.
5 Baumgartner and Hersom[8] for the examination of low and medium-acid canned food (above pH 4.5) for 'flat-sour' thermophiles, mesophilic aerobes, and facultative anaerobes.

Bashford[9] reported that the addition of 0.5–1% of meat extract greatly improves the medium.

Townsend et al. (National Canners Association[10]) showed that some batches of bromo-cresol purple are more inhibitory than others but this variability is overcome in the Oxoid medium by stringent biological control.

Technique
The instructions given below are included only as an indication of the mode of use of Dextrose Tryptone Agar, and will vary according to the original sample and the exact purpose of the investigation. For more exact details of technique it is advisable to consult one of the standard manuals mentioned in the references.

Enumeration of Mesophiles – into each of 5 petri dishes, pipette dilutions of the sample to be tested. Cover and mix the inoculum with sterile Dextrose Tryptone Agar and incubate for 72 hours at 32°C. Count the total number of colonies, with separate totals for acid producing (yellow halo) and non-acid producing colonies.

Enumeration of 'flat-sour' Thermophiles – inoculate as above and incubate for 48 hours at 55°C. 'Flat-sour' colonies (e.g. *Bacillus stearothermophilus*) are typically round, 2–5mm in diameter, with an opaque centre, and surrounded by a yellow zone in contrast with the purple medium.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared agar plates at 2–8°C.

Quality Control
Positive control:
Bacillus stearothermophilus NCIB 8919/ATCC® 12976

Negative control:
Uninoculated medium

Precautions
Incubation at 55°C must be carried out under humid conditions e.g. wrapped dishes or in a high humidity environment.

References

1 Williams O. B. (1936) *Food Res. 1(3) 217–221.*
2 Cameron E. J. (1936) *J. Assoc. Official Agr. Chem. 19. 433–438.*
3 Association of Official Analytical Chemists (1978) *Bacteriological Analytical Manual 5th Edn. AOAC Washington DC.*
4 Tanner F. W. (1944) *'The Microbiology of Foods' 2nd ed., Garrard Press, Champaers pp. 762–763 and 1127–1128.*
5 American Public Health Association (1972) *Standard Methods for the Examination of Dairy Products. 13th Edn. APHA. Washington DC.*
6 National Canners Association (1968) *Laboratory Manual for Food Canners and Processors. Vol.1. p 13.*
7 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA. Washington DC.*
8 Baumgartner J. G. and Hersom A. C. (1956) *'Canned Foods' 4th ed., Churchill Ltd., London, pp. 229–230 and 247.*
9 Bashford T. E. (1948) *Personal Communication.*
10 National Canners Association (1954) *'A Laboratory Manual for the Canning Industry' 1st ed., National Canners Association, Washington.*

DEXTROSE TRYPTONE BROTH

Code: CM73

A liquid medium for the bacteriological examination of canned foods etc. Acid producing organisms such as 'flat-sour' thermophiles change the colour of the medium from purple to yellow.

Formula	gm/litre
Tryptone	10.0
Dextrose	5.0
Bromocresol purple	0.04
pH 6.9 ± 0.2	

Directions
Add 15g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Dextrose Tryptone Broth is widely recommended for the aerobic cultivation and detection of many different organisms causing spoilage in canned foods and other products.

The American Public Health Association[1] and Baumgartner and Hersom[2] recommended this formulation for the bacteriological examination of low and medium-acid canned foods (pH 4.5 and above). Both methods include inoculation of 10ml amounts of the broth with one or two grams of the food product. For food products in this pH range, the suggested procedure is aerobic cultivation in Dextrose Tryptone Broth in parallel with anaerobic cultivation in other media; Liver Broth CM77 is most suitable for this purpose. Duplicate sets of tubes are incubated at 35°C

and at 55°C. Organisms which produce acid from dextrose, such as *Bacillus stearothermophilus* and other 'flat-sour' organisms, are detected by the colour change of the medium from purple to yellow.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared broth below 25°C.

Quality Control
Positive control:
Bacillus stearothermophilus NCIB 8919

Negative control:
Uninoculated medium

References

1 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Washington DC.*

2 Baumgartner J. G. and Hersom A. C. (1956) *'Canned Foods' 4th ed., Churchill Ltd. London, pp. 229–230 and 247.*

DIAGNOSTIC SENSITIVITY TEST AGAR (DST AGAR)

Code: CM261

A susceptibility test agar for antimicrobial testing.

Formula	gm/litre
Proteose peptone	10.0
Veal infusion solids	10.0
Glucose	2.0
Sodium chloride	3.0
Disodium phosphate	2.0
Sodium acetate	1.0
Adenine sulphate	0.01
Guanine hydrochloride	0.01
Uracil	0.01
Xanthine	0.01
Aneurine	0.00002
Agar	12.0
pH 7.4 $\pm$ 0.2	

Directions
Add 40g to 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

For blood agar, cool the base to 50°C and add 7% of Defibrinated Horse Blood SR50. Mix with gentle rotation and pour into petri dishes (12ml for a 9cm dish) or other containers. RECONSTITUTION AND MIXING SHOULD BE PERFORMED IN A FLASK AT LEAST 2.5 TIMES THE VOLUME OF MEDIUM TO ENSURE ADEQUATE AERATION OF THE BLOOD.

Description
Diagnostic Sensitivity Test Agar was developed in Oxoid as a dual purpose medium which would satisfy both diagnostic and susceptibility requirements.

The diagnostic role was supported by the nutritional amino-acid base with glucose to encourage early growth. The inclusion of the buffers (disodium phosphate and sodium acetate) helped prevent excessive movements of pH values which could result from utilisation of glucose or amino-acids. Such pH movements would interfere with haemolytic reactions[1] and the MIC values of pH-susceptible antimicrobials[2].

Long before the mechanisms of folate antagonism had been discovered, the addition of the bases adenine, guanine, uracil and xanthine were shown to improve the performance of the medium as an antimicrobial test medium.

Aneurine, added as a general purpose vitamin, improved the growth of several organisms especially staphylococci.

The agar used in the formulation has been specially processed to allow unimpeded diffusion of antimicrobials from discs[3].

DSTA CM261 is now primarily used for susceptibility tests and its role in diagnostic microbiology i.e. the primary isolation of organisms from clinical samples, has diminished.

An essential requirement for satisfactory antimicrobial susceptibility media is that the reactive levels of thymidine and thymine must be sufficiently reduced to avoid antagonism of trimethoprim and sulphonamides[4].

DSTA meets this requirement and in the presence of lysed horse blood (or defibrinated horse blood if the plates are stored long enough to allow some lysis of the erythrocytes) the level of thymidine will be further reduced. This is caused by the action of the enzyme *thymidine phosphorylase* which is released from lysed horse erythrocytes[5]. Thymidine is an essential growth factor for thymidine-dependent organisms and they will not grow in its absence or they will grow poorly in media containing reduced levels[6]. It is important that users of DSTA are aware of this limitation of thymidine which now exists in the medium and the effect it will have on a small proportion of organisms.

Details of the function of the medium and the methodology used for antimicrobial susceptibility tests are discussed in the Section 'Susceptibility Testing'.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared agar plates at 2–8°C.

Quality Control
Positive control:
Streptococcus pneumoniae ATCC® 6303

with blood
Neisseria meningitidis ATCC® 13090
Staphylococcus aureus ATCC® 25923

without blood
Enterococcus feacalis ATCC® 29212
Pseudomonas aeruginosa ATCC® 27853

Negative control:
Uninoculated medium

Precautions
Diagnostic Sensitivity Test Agar has reduced thymidine activity and this will affect its performance as a primary isolation medium.

References

1 Expert Committee on Antibiotics (1961) *World Health Organization Technical Report Series No.210. WHO, Geneva.*
2 Bechtle R. M. and Scherr G. H. (1958) *Antibiotics and Chemotherapy 8(12). 599–606.*
3 Marshall J. H. and Kelsey J. C. (1960) *J. Hyg., Camb. 58. 367–372.*
4 Ferone R., Bushby S. R. M., Burchall J.J., Moore W.D. and Smith D. (1975) *Antimicrob. Agents Chemotherap. 7. 91–98.*
5 Ferguson R. W. and Weissfeld A. S. (1984) *J. Clin. Microbiol. 19. 85–86.*
6 Stokes E. J. and Ridgway G. L. (1980) *'Clinical Bacteriology' 5th Edn. Arnold. London. p.54.*

DICHLORAN-GLYCEROL (DG18) AGAR BASE

Code: CM729

A selective low water activity (a_w) medium for xerophilic moulds from dried and semi-dried foods.

Formula	gm/litre
Peptone	5.0
Glucose	10.0
Potassium dihydrogen phosphate	1.0
Magnesium sulphate	0.5
Dichloran	0.002
Agar	15.0
Final pH 5.6 $\pm$ 0.2	

CHLORAMPHENICOL SELECTIVE SUPPLEMENT

Code: SR78

Vial contents (each vial is sufficient for 500ml of medium)

Chloramphenicol	50mg

Directions
Suspend 15.75g in 500ml of distilled water and heat to dissolve completely. Add 110g of Glycerol (Analytical Reagent grade). Rehydrate 1 vial of Chloramphenicol Supplement SR78 as directed and add to the DG18 Agar Base. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C, mix well and pour into sterile petri dishes.

Description
Dichloran-Glycerol (DG18) Agar Base CM729 is based on the formulation described by Hocking and Pitt[1] and is recommended for the enumeration and isolation of xerophilic moulds from dried and semi-dried foods. Examples of these are dried fruits, spices, confectionery, cereals, nuts and dried meat and fish products.

The medium formulation contains glycerol at 16% (w/w) which lowers the water activity (a_w) from 0.999 to 0.95. Glycerol was chosen because of advantages it showed over sodium chloride and sugars which have traditionally been used to formulate media of reduced a_w[1]. The medium also contains dichloran which inhibits spreading of mucoraceous fungi and restricts the colony size of other genera. This restrictive characteristic makes the medium especially suitable for enumeration because it allows unobscured growth of organisms that ordinarily form small colonies.

A modification to the formula has been described in which the addition of Triton-X to DG18 agar increases the inhibition of vigorously-spreading fungi[2].

In a comparative study carried out between DG18 and DRBC, (a medium of higher a_w) greater recovery of xerophilic moulds was achieved on the DG18 medium[1]. In this study it was found that two of the fungi commonly isolated from dried foods in high numbers, *Aspergillus penicilloides* and *Wallemia sebi*, grow very poorly or not at all on DRBC.

Technique
1 Prepare the DG18 medium as directed using CM729, SR78 and glycerol.
2 Process the food sample in a Seward 'Stomacher' adding 40g to 200ml of 0.1% peptone water. For powdered products shake periodically for 30 minutes with 0.1% peptone water.
3 Dilute the sample 1:10 in 0.1% peptone water.
4 Surface plate 0.1ml of the prepared sample per plate.
5 Incubate at 25°C and examine after 4, 5 and 6 days.
6 Report as number of xerophilic colonies per gram of food.

Further experience with this medium has shown it to be a good general purpose medium. In a collaborative exercise in Holland the DG18 medium gave the best results for yeasts and moulds isolated from foodstuffs[2].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control
Positive control:
Mucor racemosus ATCC® 42647
Saccharomyces cerevisiae ATCC® 9763

Negative control:
Escherichia coli ATCC® 25922
Bacillus subtilis ATCC® 6633

Precautions
The dichloran compound used in this medium is Botran® 2,6-Dichloro-4-Nitro-Analine (CAS: 99–30–9).

References

1 Hocking A. D. and Pitt J. I. (1980) *J. Appl. & Env. Microbiol. 39. 488–492.*
2 Beuchat L. R. and Hwang C. A. (1996) *Int. J. Food Microbiol. 29. 161–166.*
3 Beckers H. J., Boer E., van Eikelenboom C., Hartog B. J., Kuik D., Mol N., Nooitgedagt A. J., Northolt M. O. and Samson R. A. (1982) *Intern. Stand. Org. Document ISO/TC34/SC9/N151.*

DNASE AGAR

Code: CM321

For the detection of microbial deoxyribonuclease enzymes, particularly from staphylococci.

Formula	gm/litre
Tryptose	20.0
Deoxyribonucleic acid	2.0
Sodium chloride	5.0
Agar	12.0
pH 7.3 ± 0.2	

Directions
Suspend 39g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Weckman & Catlin[1] suggested that DNase activity could be used to identify pathogenic staphylococci after they had established a close correlation with coagulase production. Jeffries et al.[2] incorporated DNA in the agar medium to provide a simple method of detecting DNase activity. Organisms are streaked on to the surface of the agar medium and incubated. The growth on the surface of the agar is then flooded with 1N hydrochloric acid. Polymerised DNA precipitates in the presence of 1N HCl and makes the medium opaque. If the organisms produce DNase enzymes, in sufficient quantity to hydrolyse the DNA, then clear zones are seen around the colonies.

Good correlation was shown between DNase production and coagulase activity when testing *Staphylococcus aureus* strains from clinical samples[2,3,4]. Both *Staph. aureus* and *Staph. epidermis* produce extracellular DNase[5,6,7] but *Staph. aureus* produces greater quantities[1,7].

A modification of the medium is to add mannitol (1% w/v) and phenol red or bromothymol blue (0.0025% w/v) as an indicator of mannitol fermentation[9]. The pH reaction around the colonies must be read before the plate is flooded with acid.

The DNase reaction helps in the differentiation and identification of non-pigmented *Serratia marcescens*[8] (positive DNase reaction) from *Klebsiella-Enterobacter* (negative DNase reaction).

Normal HCl is bactericidal and the organisms cannot be recovered from the surface of the agar after flooding. The incorporation of dyes into the medium which can distinguish hydrolysis of DNA is a further modification which avoids the use of acid. Toluidine blue[8] and methyl green[10] form coloured complexes with polymerised DNA; these colours change as the DNA is hydrolysed.

It should be noted that toluidine blue inhibits Gram positive organisms and it is used to detect DNase production by the Enterobacteriaceae. It has been used with ampicillin (30mg/litre) to demonstrate DNase production by *Aeromonas hydrophila* from faeces[11].

Technique
Inoculate the plates by spotting the organism onto the surface of the agar so that a thick plaque of growth is evident after 18 hours incubation.

Examine plates for colour changes in or around the colonies if mannitol/indicator or dyes have been added to the medium. In the absence of dyes, flood the plates with 1N HCl and allow them to stand on the bench (lids uppermost) for a few minutes. Look for zones of clearing around the colonies.

Appearance of colonies with media modifications

1	Mannitol/pH indicator:	
	Yellow, with yellow zones	Mannitol +
	Same colour as medium	Mannitol –
2	Toluidine blue:	
	Pink zones in blue medium	DNase +
	No zones	DNase –
3	Methyl green:	
	Almost colourless zones	DNase +
	No zones	DNase –
4	Acid flood:	
	Well defined clear zones	DNase +
	No clear zones	DNase –

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium at 2–8°C.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923
Serratia marcescens ATCC® 8100

Negative control:
Staph. epidermidis ATCC® 12228
Klebsiella pneumoniae ATCC® 13883

Precautions
The DNase reaction for staphylococci is an indication of pathogenicity, it cannot be used as the sole criterion for identification.

Small zones of clearing may be caused by other enzymes or organic acid production[7].

Other organisms than staphylococci, serratia and aeromonads can produce DNases.

Once the hydrochloric acid has been applied to the medium the plate must be read within a few minutes and further testing cannot be carried out by re-incubation.

The methyl green must be purified by extraction with chloroform[10].

Toluidine blue varies in performance according to source.

Merck Toluidine blue 1273 is satisfactory. Note that this dye cannot be used for Gram positive organisms.

References
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DRBC AGAR BASE

Code: CM727

Dichloran Rose-Bengal Chloramphenicol Agar is a selective medium for yeasts and moulds associated with food spoilage.

Formula	gm/litre
Peptone	5.0
Glucose	10.0
Potassium dihydrogen phosphate	1.0
Magnesium sulphate	0.5
Dichloran	0.002
Rose-Bengal	0.025
Agar	15.0
pH 5.6 $\pm$ 0.2	

CHLORAMPHENICOL SELECTIVE SUPPLEMENT

Code: SR78

Vial contents (each vial is sufficient for 500ml of medium)

Chloramphenicol	50.mg

Directions
Suspend 15.75g in 500ml of distilled water and heat to dissolve completely. Rehydrate 1 vial of Chloramphenicol Supplement SR78 as directed and add to the DRBC Agar Base. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C, mix well and pour into sterile petri dishes.

Description
Dichloran Rose-Bengal Chloramphenicol Medium (DRBC) CM727 is based on the formulation described by King et al.[1,2], and is recommended as a selective medium for the isolation and enumeration of yeasts and moulds that are of significance in food spoilage.

DRBC is a modification of Rose-Bengal Chloramphenicol Medium[3] and differs as follows: pH is lowered to 5.6, the Rose-Bengal content is reduced by 50% and Dichloran is added.

The cumulative effect of these modifications is to further inhibit bacterial growth, inhibit spreading moulds such as *Rhizopus* and *Mucor* and make the medium capable of supporting the growth of those species that cannot be isolated on Rose-Bengal Chloramphenicol Agar or acidified Potato Dextrose Agar[1]. The inhibition of spreading moulds and the general restriction of colony size results in improved enumeration and detection of mycotoxigenic moulds and other species of significance in food spoilage[6].

In a collaborative exercise in the UK between nine laboratories, in which mould and yeast counts were made on different samples of food and feed, DBRC came out best of the five different media tested[7].

Rose-Bengal Chloramphenicol Agar should be used in addition where it is necessary to gain an overall impression of the fungal flora, including spreading types, when the use of DRBC Agar alone would inhibit these.

The reduced pH of DRBC Agar increases the inhibition of yeasts by Rose-Bengal[1] and the use of Rose-Bengal Chloramphenicol Agar (pH of 7.2) in parallel should be considered where it is necessary to enumerate yeasts in the presence of moulds.

Technique
1 Prepare the DRBC Medium as directed using CM727 and SR78.
2 Add 40ml of the food sample to 200ml of 0.1% peptone water and process in a Seward 'Stomacher' for 30 seconds[4] or alternatively weigh into 0.1% peptone water and leave for 30 minutes shaking periodically[5].
3 Inoculate 0.1ml of the prepared sample on the medium surface.
4 Incubate the plates at 25°C and examine after 3, 4 and 5 days.
5 Report as number of colonies per gram of food.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium at 2–8°C.

Quality Control
Positive control:
Mucor racemosus ATCC® 42647
Saccharomyces cerevisiae ATCC® 9763

Negative control:
Escherichia coli ATCC® 25922
Bacillus subtilis ATCC® 6633

Precautions
ROSE-BENGAL PHOTO-OXIDISES TO FORM TOXIC COMPOUNDS. STORE PLATES OF THE MEDIUM IN THE DARK AND AVOID EXPOSURE TO LIGHT[8].

Some strains of fungi may be inhibited on this medium.

The dichloran compound used in this medium is Botran® 2,6-Dichloro-4-Nitro-Analine (CAS: 99–30–9).

References
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EDWARDS MEDIUM (MODIFIED)

Code: CM27

A selective medium for the rapid isolation of Streptococcus agalactiae and other streptococci involved in bovine mastitis.

Formula	gm/litre
'Lab-Lemco' powder	10.0
Peptone	10.0
Aesculin	1.0
Sodium chloride	5.0
Crystal violet	0.0013
Thallous sulphate	0.33
Agar	15.0
pH 7.4 $\pm$ 0.2	

POISON – Contains Thallium Salt.

Directions
Suspend 41g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 115°C for 20 minutes. Cool to 50°C, add 5 to 7% of sterile bovine or sheep blood, mix well and pour plates.

Description
A selective medium for the rapid isolation of *Streptococcus agalactiae* and other streptococci involved in bovine mastitis.

Crystal violet or gentian violet and thallium salts have long been used in selective media for streptococci. Haxthausen[1] employed a selective crystal violet medium for the isolation of skin streptococci. Bryan[2] using gentian violet blood agar, found that the growth of saprophytic milk bacteria was prevented whilst that of streptococci was unaffected. Edwards[3] employed a crystal violet aesculin blood agar for the cultural diagnosis of bovine mastitis, whilst McKenzie[4] used a medium containing thallium acetate for the same purpose.

Hauge et al.[5] described a composite medium containing all the components of modified Edwards Medium.

Aesculin differentiates the negative *Streptococcus agalactiae* (blue colonies) from aesculin-positive Group D streptococci (black colonies).

Technique
Inoculate the surface of the medium with centrifuged deposits from milk samples and incubate at 35°C. Look for pale blue colonies which should then be subcultured for further identification tests.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C. and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control
Positive control:
Streptococcus agalactiae ATCC® 13813
Enterococcus faecalis ATCC® 29212

Negative control:
Escherichia coli ATCC® 25922
Staphylococcus epidermidis ATCC® 12228

References
1 Haxsthausen H. (1927) *Ann. Derm. Syph. 8. 201.*
2 Bryan C. S. (1932) *Am. J. Pub. Hlth. 22. 749.*
3 Edwards S. J. (1933) *J. Comp. Path. Therap. 46 211–217.*
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EE BROTH

Code: CM317

An enrichment medium for Enterobacteriaceae in the bacteriological examination of foods.

Formula	gm/litre
Peptone	10.0
Glucose	5.0
Disodium hydrogen phosphate anhyd.	6.45
Potassium dihydrogen phosphate	2.0
Ox Bile purified	20.0
Brilliant green	0.0135
pH 7.2 $\pm$ 0.2	

Directions
Add 43.5g to 1 litre of distilled water. Distribute 100ml quantities in 250ml flasks and heat at 100°C for 30 minutes only. Cool rapidly in cold running water. This medium is heat sensitive. DO NOT AUTOCLAVE.

Description
EE Broth (Buffered glucose – Brilliant Green-bile broth) is recommended as an enrichment medium for *Enterobateriaceae* in the bacteriological examination of foods[1] and animal feed stuffs[2]. This medium is more inhibitory to non-Enterobacteriaceae than other non-selective media e.g. Mannitol broth[3] or Lactose broth[4] by virtue of the presence of brilliant green and bile salts in the preparation.

The enumeration of Enterobacteriaceae is of great importance in monitoring the sanitary quality of food and drugs but the reliability of the methods used depends upon resuscitation of damaged cells. Such weakened cells may arise from exposure to dehydration, low pH and other unfavourable conditions[5].

Incubation for 2 hours in well-aerated Tryptone Soya Broth CM129 at 25°C should precede enrichment in

EE Broth. This procedure is recommended for dried foods[6], animal feeds[7] and semi-preserved foods[8]. Occasionally, with a particular dry product, a longer incubation period is necessary but never over eight hours of resuscitation.

Oxoid EE Broth was formulated to overcome the unsatisfactory effects of inhibition on small numbers of Enterobacteriaceae cells due to bile salt variations. The inclusion of purified ox bile eliminated these problems and a preliminary assay can be used to check growth by inoculating approximately one viable cell per medium unit[9,10].

For the bacteriological evaluation of processed foods the entire Enterobacteriaceae group can be used as indicator organisms[10]. This will overcome the discrepancies that can arise when lactose-negative, anaerogenic lactose-positive or late lactose fermenting Enterobacteria are present but are missed by the standard 'coli-aerogenes' tests. To overcome these problems lactose media have been replaced by those containing glucose. Mossel et al.[1] cited several examples in the literature which referred to various foods contaminated with salmonellae, although results for coliforms were negative. A later example quoted by Mossel[9] involved an outbreak of diarrhoea caused by French mould-fermented soft cheese contaminated by *Escherichia coli* serotype 0124. This organism is lactose-negative and therefore was not detected in coliform tests but only recognised when the commodity was tested for Enterobacteriaceae since it fermented glucose rapidly.

EE Broth should be used as an enrichment broth in conjunction with Violet Red Bile Glucose Agar CM485. When specific organisms, rather than Enterobacteriaceae in general, are required subcultures must be made onto lactose differential media e.g. Desoxycholate Citrate Agar CM35, Brilliant Green Agar CM329, or MacConkey Agar CM7 for the detection of lactose-negative or delayed organisms.

Sample size should not be less than 10 grams to yield the organisms being sought.

Technique

1 Resuscitate debilitated cells by incubating 1:10 dilutions of the food samples under investigation in Tryptone Soya Broth CM129 at 25°C for 2–8 hours. The fluid layer should not be much deeper than one centimetre. Shake the flask to disperse the contents alternately in clockwise and anti-clockwise directions for 30 seconds on three successive occasions.

2 After the period of time necessary for resuscitation, ten-fold volumes of EE Broth are added to the resuscitated suspensions.

3 Shake to disperse as above. For large samples it is desirable to add the resuscitation medium containing the product under examination, to equal volumes of double strength EE Broth.

4 Incubate at :
   44°C for 18 hours for thermotrophic bacteria
   32°C for 24/48 hours for mesophilic bacteria
   4°C for 10 days for psychrotrophic bacteria
   depending on the groups of Enterobacteriaceae sought.

5 Examine the tubes of broth and look for turbidity with some change of colour towards yellowish-green for presumptive evidence of Enterobacteriaceae.

6 Subcultures can be made on to Violet Red Bile Glucose Agar CM485 or on to lactose-containing media for confirmation of LF or NLF status. Further tests must be made to confirm the identity of the isolate.

Storage conditions and Shelf life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared broth at 2–8°C.

Quality Control

Positive control:
Yersinia enterocolitica NCTC 10460
Escherichia coli ATCC® 25922

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions

Avoid overheating the medium, especially the double-strength broth.

References

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ENDO AGAR BASE

Code: CM479

A modified medium requiring the addition of basic fuchsin to form Endo Agar.

Formula	gm/litre
Peptone	10.0
Lactose	10.0
Di-potassium phosphate	3.5
Sodium sulphite	2.5
Agar	10.0
pH 7.5 ± 0.2	

Directions
Suspend 36g in 1 litre of distilled water. Add 4ml (or as directed by the supplier) of a 10% w/v alcoholic solution of basic fuchsin BR50 (95% Ethyl Alcohol). Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring.

BASIC FUCHSIN IS A POTENTIAL CARCINOGEN AND CARE SHOULD BE TAKEN TO AVOID INHALATION OF THE POWDERED DYE AND CONTAMINATION OF THE SKIN.

Plates should be stored in the dark to preserve their pale pink colour.

Description
Endo Agar is a long established medium which was originally devised for the isolation of the typhoid bacillus. More reliable media for this purpose have since been evolved, and the medium is now used for the differentiation of lactose fermenting and non-lactose fermenting intestinal organisms, particularly during confirmation of the presumptive test for coliforms. Production of both acid and aldehyde by lactose fermenting organisms, such as *Escherichia coli*, gives rise to the characteristic red coloration of the colony and the surrounding medium.

Technique
For the confirmation of presumptive tests with liquid media, subculture tubes showing gas, or acid and gas formation, onto an Endo Agar plate. Incubate for 24 hours at 35°C.

Lactose fermenting coliforms (e.g. *Escherichia coli*) give rise to deep red colonies which colour the surrounding medium and possess a golden metallic sheen.

Non-lactose fermenters form colourless translucent colonies, against the pink to colourless medium.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C away from light.

Quality Control
Positive control:
Escherichia coli ATCC® 25922
Enterobacter aerogenes ATCC® 13048
Proteus vulgaris ATCC® 13315

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions
Weigh out the basic fuchsin (BR50) in a fume cupboard and avoid inhalation of the powder or contamination of the skin.

Keep the prepared medium away from light to avoid photo-oxidation.

Endo Agar is quoted by the American Public Health Association as a 'Standard Methods' medium for use in water[1] and dairy products[2]. Windle Taylor[3] recommended the medium for the isolation and differentiation of coli-aerogenes bacteria from water.

References

1 American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington DC.*
2 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.*
3 Windle Taylor E. (1958) *'The Examination of Waters and Water Supplies' 7th ed., Churchill Ltd., London, pp. 417. 440–441, 780–781.*

EOSIN METHYLENE BLUE AGAR (MODIFIED) LEVINE

Code: CM69

An isolation medium for the differentiation of the Enterobacteriaceae.

Formula	gm/litre
Peptone	10.0
Lactose	10.0
Dipotassium hydrogen phosphate	2.0
Eosin Y	0.4
Methylene blue	0.065
Agar	15.0
pH 6.8 ± 0.2	

Directions
Suspend 37.5g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 60°C and shake the medium in order to oxidise the methylene blue (i.e. restore its blue colour) and to suspend the precipitate which is an essential part of the medium.

Description
This versatile medium, modified by Levine[1,2], is used for the differentiation of *Escherichia coli* and *Enterobacteria aerogenes*, for the rapid identification of *Candida albicans*, and for the identification of coagulase-positive staphylococci.

The medium is prepared to the formula specified by the APHA[3,4,5,6] for the detection and differentiation of the coliform group of organisms[7,8].

Weld[9,10] proposed the use of Levine eosin methylene blue agar, with added chlortetracycline hydrochloride for the rapid identification of *Candida albicans* in clinical materials. A positive identification of *Candida albicans* could be made after 24 to 48 hours incubation at 37°C in 10% carbon dioxide from faeces, oral and vaginal secretions, and nail or skin scrapings. Vogel and Moses[11] confirmed the reliability of Weld's

method for the relatively rapid identification of *C. albicans* in sputum. They found that use of eosin methylene blue agar was just as reliable as more conventional methods for the identification of this organism in sputum. In addition, the medium provided a means for the identification of several Gram-negative genera. Doupagne[12] also investigated the use of the Levine medium under tropical conditions.

Haley and Stonerod[13] found that Weld's method was variable so that Walker and Huppert[14] advocated the use of corn meal agar and a rapid fermentation test in addition to the Levine medium. Using the combined rapid technique they were able to obtain results within 48 to 72 hours.

Subsequent to the findings of Vogel and Moses[11], Menolasino et al.[15] used Levine eosin methylene blue agar for the identification of coagulase-positive staphylococci which grew as characteristic colourless, pin-point colonies. The Levine medium was more efficient than tellurite glycine agar and showed good correlation with the plasma coagulase test.

Colonial Characteristics

Escherichia coli – isolated colonies, 2–3mm diameter, with little tendency to confluent growth, exhibiting a greenish metallic sheen by reflected light and dark purple centres by transmitted light.

Enterobacter aerogenes – 4–6mm diameter, raised and mucoid colonies, tending to become confluent, metallic sheen usually absent, grey-brown centres by transmitted light.

Non-lactose fermenting intestinal pathogens – translucent and colourless.

Candida albicans – after 24 to 48 hours at 35°C in 10% carbon dioxide 'spidery' or 'feathery' colonies. Other *Candida* species produce smooth yeast-like colonies. Since a typical appearance is variable it is advisable to use a combined method such as that of Walker and Huppert[14].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C away from light.

Quality Control
Positive control:
Escherichia coli ATCC® 25922
Enterobacter aerogenes ATCC® 13048
Staphylococcus aureus ATCC® 25923

Negative control:
Uninoculated medium

Precautions
Further tests are required to confirm the presumptive identity of organisms isolated on this medium. Some strains of *Salmonella* and *Shigella* species will not grow in the presence of eosin and methylene blue. Store the medium away from light to prevent photo-oxidation.

References

1 Levine M. (1918) *J. Infect. Dis. 23. 43–47.*
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FRASER BROTH

Code: CM895

A secondary selective diagnostic enrichment medium for the isolation of Listeria spp. from food and environmental specimens.

Formula	gm/litre
Proteose peptone	5.0
Tryptone	5.0
'Lab-Lemco' powder	5.0
Yeast extract	5.0
Sodium chloride	20.0
Disodium hydrogen phosphate	12.0
Potassium dihydrogen phosphate	1.35
Aesculin	1.0
Lithium chloride	3.0
pH 7.2 $\pm$ 0.2	

FRASER SUPPLEMENT

Code: SR156

Vial contents (each vial is sufficient to supplement 500ml of medium)

Ferric ammonium citrate	0.25g
Nalidixic acid	10.0mg
Acriflavine hydrochloride	12.5mg

Directions
Suspend 28.7g in 500ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of Fraser Selective Supplement SR156 reconstituted with 5ml of ethanol/sterile water (1:1). Mix well and distribute into sterile containers.

Description
Fraser medium is a modification of the USDA-FSIS (United States Department of Agriculture-Food Safety Inspection Service) UVM secondary enrichment broth and is based on the formula described by Fraser and Sperber[1]. It contains ferric ammonium citrate and lithium chloride. Blackening of the medium is presumptive evidence of the presence of Listeria. Contrary to early indications, cultures which do not blacken cannot be assumed to be Listeria-free. All Fraser Broth enrichment cultures should be sub-cultured to plating medium.

The medium is intended for the isolation of *Listeria* spp. from food and environmental samples when used as the secondary enrichment medium in the USDA-FSIS methodology for Listeria isolation.

It is generally accepted that the USDA-FSIS two stage enrichment method employing UVM primary and secondary enrichment broths is the most suitable for the examination of meat products. Fraser Broth has proven to be remarkably accurate in detecting *Listeria* spp. in food and environmental samples[1,2].

All *Listeria* spp. hydrolyse aesculin to aesculetin. Aesculetin reacts with ferric ions which results in blackening. Another possible advantage to the addition of ferric ammonium citrate is that it has been shown that ferric ions enhance the growth of *L. monocytogenes*[3].

Lithium chloride is included in the medium to inhibit the growth of enterococci which can also hydrolyse aesculin.

Care must be taken when using Fraser Broth with DNA probe methodology because the high salt content of the medium may have an inhibitory effect on detection[4].

Technique
1 Inoculate 10ml of Fraser Broth with 0.1ml of the primary enrichment broth (i.e. FDA or UVM I enrichment broth) which has been incubated for 20 to 24 hours.
2 Incubate at 35°C for 26 ± 2 hours in air.
3 Compare each inoculated tube to an inoculated control against a white background. Tubes that darken or turn black should be subcultured on to Oxford Medium, Modified Oxford Medium (MOX) or PALCAM Medium. Tubes that retain the original yellow colour should also be inoculated on plating media and confirmed as free from *Listeria* spp. before discarding.

It should be emphasised that the incubation period should be controlled. Fraser Medium should be incubated for 26 ± 2 hours to ensure at least 24 hours incubation period to permit the development of the black colour.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the selective supplement in the dark at 2°C to 8°C and use before the expiry date on the label.

The prepared medium may be stored for up to 2 weeks at 2°C to 8°C.

Quality Control
Positive control:
Listeria monocytogenes ATCC® 19117

Negative control:
Enterococcus faecalis ATCC® 29212

References

1 Fraser J.A. and Sperber W.H. (1988) *J. Food Protect. 51, No.10, 762–765.*
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HALF FRASER SUPPLEMENT

Code: SR166E

Please see 4–6.

GARDNERELLA VAGINALIS SELECTIVE MEDIUM

For the isolation of Gardnerella vaginalis.

BASE MEDIUM

COLUMBIA BLOOD AGAR BASE

Code: CM331

Formula	gm/litre
Special peptone	23.0
Starch	1.0
Sodium chloride	5.0
Agar	10.0
pH 7.3 ± 0.2	

Directions
Suspend 19.5g in 450ml of distilled water. Boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add 50ml of sterile human, rabbit or horse blood and the rehydrated contents of 1 vial of Gardnerella Vaginalis Selective Supplement SR119.

GARDNERELLA VAGINALIS SELECTIVE SUPPLEMENT

Code: SR119

Vial contents (each vial is sufficient for 500ml of medium)

Gentamicin sulphate	2mg
Nalidixic acid	15mg
Amphotericin B	1mg

Directions
To rehydrate one vial of Gardnerella Vaginalis Selective Supplement SR119 add 2ml of ethanol and sterile distilled water (1:1).

Description
Gardnerella Vaginalis Selective Supplement SR119, is based on the formulation of Ison et al.[1] and is recommended for the selective isolation of *G. vaginalis* from the vaginal discharge of patients with symptoms

of Non-specific Vaginitis (NSV). The symptoms of this mild condition prior to the isolation of the aetiological agent(s) are:

1 The absence of recognised pathogens.
2 Foul smelling discharge.
3 pH greater than 4.5.
4 Release of 'fish' odour on the addition of potassium hydroxide (10%) to the discharge.
5 The presence of 'clue' cells in prepared wet mounts (these are epithelial cells with a characteristic stippled or granular appearance caused by Gram variable bacilli adhering to the cell surface).

Several media and techniques have been described for the isolation of *G. vaginalis*. The Oxoid Gardnerella Vaginalis Selective Medium can be used for the surface inoculation technique or the double layer technique[2].

With added human blood or rabbit blood[3], a beta-haemolytic reaction is exhibited by *G. vaginalis*. This can be used as a preliminary diagnosis feature[1]. The addition of 'Tween 80' (0.02% v/v) to the medium containing human blood has been found to give enhanced beta-haemolytic zones[4,5].

G. vaginalis is a Gram variable, small, pleomorphic bacillus which forms 0.25–0.44mm diameter colonies producing beta-haemolysis on medium containing human blood.

Technique

Surface Inoculation Method (Isolation)

1 Prepare the selective medium from Columbia Blood Agar Base CM331, Gardnerella Vaginalis Selective Supplement SR119 and defibrinated Horse Blood SR51, according to the directions. To demonstrate the characteristic haemolysis with human or rabbit blood, substitute human or rabbit for horse blood when preparing the medium.
2 Using a swab inoculate the vaginal discharge on to the medium.
3 Incubate, at 35°C for 48 hours in an atmosphere containing 7% carbon dioxide[6].
4 Carry out confirmatory tests on all colonies from medium containing horse blood and on beta-haemolytic colonies from medium containing human blood or rabbit blood.

Double Layer Method (Isolation and Presumptive identification)

1 Prepare two lots of selective medium from Columbia Blood Agar Base CM331, Gardnerella Vaginalis Selective Supplement SR119 and sterile human blood according to the directions.
2 Use one lot to prepare base medium plates and place the second lot in a water bath at 50°C.
3 Using the swab inoculate the vaginal discharge on to the surface of the prepared plates. Allow to dry at room temperature for half an hour.
4 Overlay with 5ml of the selective medium at 50°C.
5 Allow the overlay medium to set.
6 Incubate at 35°C for 48 hours in an atmosphere containing 7% carbon dioxide.
7 Carry out confirmatory tests on isolates that show a beta-haemolytic zone. Use an inoculating wire to stab through the agar overlay to reach the colonies beneath.

The following tests have been compiled from the literature and personal communication.

Test or Substrate	Test Result	% Positive
Oxidase	Negative	0
Catalase	Negative	0
Haemolysis of:		
Human blood	Positive	96[7]
Rabbit blood	Positive	96
Horse blood	Negative	some strains
Sheep blood	Negative	0[7]
Hippurate hydrolysis	Positive	92
Starch hydrolysis	Positive	90
Metronidazole (50μg)	Susceptible	90
Trimethoprim (5μg)	Susceptible	100
Sulphonamide (1000μg)	Resistant	0

Storage conditions and Shelf life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control

Positive control:
Gardnerella vaginalis ATCC® 14018

Negative control:
Escherichia coli ATCC® 25922

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GBS AGAR BASE (ISLAM)

Code: CM755

For the isolation and detection of Group B streptococci (GBS) in clinical specimens.

Formula	gm/litre
Proteose peptone	23.0
Soluble starch	5.0
Sodium dihydrogen phosphate	1.5
Di-sodium hydrogen phosphate	5.75
Agar	10.0
pH 7.5 ± 0.1	

Directions
Suspend 45.2g in 1 litre of distilled water and bring to the boil to disolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add 50ml of sterile inactivated Horse Serum*. Mix well and pour into petri dishes.

**Sterile Inactivated Horse Serum*
Hold sterile Horse Serum (Oxoid SR35) at 56°C for 30 minutes.

Description
GBS Agar Base CM755 is based on the formulation described by Islam. The medium is designed to exploit the ability of most group B streptococci (GBS) to produce orange/red pigmented colonies when incubated under anaerobic conditions.

Group B streptococci are a recognised cause of serious neonatal infection acquired from the infected mother. A review[2] of national data over an 8 year period by the Public Health Laboratory Service showed that group B streptococci accounted for 29.5% of all reports of neonatal bacterial meningitis with organisms being isolated from CSF and blood.

Group B streptococci may also be isolated from adults infected in a variety of sites.

The pigment of group B streptococci has characteristics of a carotenoid[3] and was first noted by Lancefield in 1934 in nine of twenty-four strains grown anaerobically. Modifications of media[1,4,5] have improved the proportion of pigmented strains to about 97%. Noble et al.[6] reported that in their studies 99.5% of beta-haemolytic GBS strains produced pigment. GBS Agar also supports growth of other genital bacteria that cause perinatal infections[1], e.g. anaerobic streptococci, *Bacteroides* and *Clostridium* species.

Colonies of group B streptococci are 0.5 to 1mm in diameter, round, entire and pigmented orange/red after 24 to 48 hours anaerobic incubation. Other organisms that can grow on this medium do not produce the orange/red pigment.

de la Rosa et al.[7] demonstrated the pigment-enhancing effect of trimethoprim/sulphonamides added to their medium. Work carried out in the Oxoid laboratories has shown that this pigment-enhancing effect can also be demonstrated around a sulphonamide disc placed on the inoculated plate. Standard discs of SF300 or SF500 can be used for this purpose. No inhibition of growth occurs and the pigment effect is clearly seen over a radius of 10–20mm.

Technique
1 Swabs should be collected into Stuart's Transport Medium CM111 and processed within 1/2–2 hours of collection[8].
2 Inoculate the swab on to the surface of GBS Agar.
3 If desired, apply a disc containing 300 or 500μg of Sulphafurazole on to an area of the plate where growth can be expected to be moderately profuse. Such discs are available from Oxoid with product code SF300 or SF500.
4 Incubate the plates anaerobically at 35°C for 24 to 48 hours. The Oxoid Anaerobic system with a Gas Generating Kit SR38 is recommended.
5 Report all orange/red pigmented colonies as presumptive group B streptococcus.
6 Confirm identity with the Oxoid Streptococcal Grouping Kit DR585.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C away from light.

Quality Control
Positive control:
Streptococcus agalactiae ATCC® 13813

Negative control:
Enterococcus faecalis ATCC® 29212

Precautions
The medium must be at its correct pH value to ensure good pigmentation.

Some strains of Group B streptococci do not produce pigmented colonies.

Do not hold the molten medium longer than necessary to fill out the dishes.

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GC SELECTIVE MEDIA

Isolation techniques for Pathogenic Neisseria.

NEISSERIA ISOLATION MEDIA AND TECHNIQUES

Introduction
The probability of success in the isolation and identification of pathogenic Neisseria from clinical specimens is related to five factors:

1 The amount of care taken in obtaining good specimens, their transport to the laboratory and correct inoculation on culture medium.
2 The provision of a culture medium capable of growing small inocula of demanding strains.
3 The provision of optimal incubation temperature and gaseous environment.
4 The inclusion of selective agents in the medium which are capable of preventing overgrowth of commensal organisms but which will not inhibit the growth of the species required.
5 Confirmatory tests to identify the species.

Importance of Accurate Diagnosis
Diagnosis of gonorrhoea is achieved by microscopic examination and cultivation of material from infected sites. Presumptive gonococci have then to be distinguished from other organisms of the *Neisseria* group which have a similar morphology and staining characteristics.

Sampling Sites
The diagnosis is achieved by examining smears and cultures from a number of sampling sites. These include the urethra and rectum of males, the urethra, cervix and rectum of females and occasionally also the ducts of Bartholin's glands. The oropharynx of both sexes may also require sampling[1,2]. Repeated examination may be necessary before a diagnosis is achieved. The calcium alginate or cotton wool swabs that are used must be of low toxicity for bacteriological purposes.

As with meningococcal infection, gonorrhoea may present as septicaemia with signs of rash, fever and/or arthritis and the responsible organism may be isolated from the blood, joints or (rarely) the cerebrospinal fluid. Ocular infections can occur in neonates and occasionally in adults.

Inoculation
The plates are inoculated by rolling the culture swab across a segment of a moist plate or preferably in a large 'Z' pattern so that an adequate area of the plate is inoculated. Streaking the plate with a sterile inoculating loop is carried out to ensure adequate dispersion of the organisms.

Media
Media used for the cultivation of gonococci are agars of high peptone and starch content enriched with fresh horse blood (lysed) or soluble haemoglobin and GC growth supplements (Yeast Autolysate Supplement SR105 and Vitox SR90) which have been shown to stimulate growth from small inocula.

Several combinations of selective antibiotics (VCNT, VCN, LCAT and VCAT) have been described that can be added to culture media in order to suppress Gram-positive and Gram-negative contaminants.

The choice of the selective supplement is dependent upon the preference of the laboratory as well as regional and strain differences of the organism.

OXOID GC AGAR BASE

Code: CM367

Oxoid GC Agar Base has been formulated to include Special Peptone L72 which is a mixture of meat and plant enzymatic digests. The presence of starch ensures that toxic metabolites produced by neisseria are absorbed. Phosphate buffers are included to prevent changes in pH due to amine production that would affect the survival of the organism.

Formula	gm/litre
Special peptone	15.0
Corn starch	1.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	4.0
Potassium dihydrogen phosphate	1.0
Agar	10.0
pH 7.2 $\pm$ 0.2	

SOLUBLE HAEMOGLOBIN POWDER

Code: L53

A specially prepared powder which will form a solution of 2% w/v in water and remain stable after sterilisation.

YEAST AUTOLYSATE GROWTH SUPPLEMENT

Code: SR105

Yeast Autolysate Supplement is a sterile lyophilised concentrate of specially prepared yeast fractions with glucose and sodium bicarbonate.

Vial contents (each vial is sufficient for 500ml of medium)

Yeast autolysate	5.0g
Glucose	0.5g
Sodium bicarbonate	0.075g

VITOX

Code: SR90/SR90B

Vitox is a sterile lyophilised concentrate of essential growth factors. Many workers prefer such a chemically defined growth supplement to yeast extracts for the supplementation of Thayer Martin Medium.

VITOX (LYOPHILISED)

Code: SR90

Contents (per vial)

Vitamin B_{12}	0.1mg
L-glutamine	100.0mg
Adenine SO_4	10.0mg
Guanine HCl	0.3mg
p-Aminobenzoic acid	0.13mg
L-cystine	11.0mg
NAD (Coenzyme 1)	2.5mg
Cocarboxylase	1.0mg
Ferric nitrate	0.2mg
Thiamine HCl	0.03mg
Cysteine HCl	259.0mg
Glucose	1.0g

VITOX HYDRATION FLUID

Code: SR90B

Contents (per vial)

Glucose	1.0g
Distilled water	10.0ml

Vitox may be used satisfactorily at a final concentration of 1% v/v in culture media (1 vial to 1,000ml of medium).

However, it was found at Oxoid Laboratories that increasing the concentation to 2% v/v (1 vial to 500ml of medium) in Thayer Martin Medium resulted in faster growth of *Neisseria gonorrhoeae*. Use at 2% v/v is therefore recommended.

STERILE SELECTIVE ANTIBIOTIC SUPPLEMENTS

The following range of Selective Antibiotic Supplements are available which can be added to prepare Thayer Martin Medium and derivatives of the New York City Medium formulation.

VCN SELECTIVE SUPPLEMENT

Code: SR101

An antibiotic supplement for the isolation of N. gonorrhoeae and N. meningitidis.

Vial contents (each vial is sufficient for 500ml of medium)

Vancomycin	1.5mg
Colistin methane sulphonate	3.75mg
Nystatin	6,250 IU

Thayer and Martin[3] described a medium to which the above antibiotics are added. This selective medium for gonococci and meningococci has been widely accepted for the primary isolation of these organisms from conspicuously contaminated sites.

VCNT SELECTIVE SUPPLEMENT

Code: SR91

An antibiotic supplement for the isolation of N. gonorrhoeae and N. meningitidis.

Vial contents (each vial is sufficient for 500ml of medium)

Vancomycin	1.5mg
Colistin methane sulphonate	3.75mg
Nystatin	6,250 IU
Trimethropin	2.5mg

Seth[4] described a modification of Thayer Martin Medium in which Trimethoprim 5μg/ml was added to the VCN antibiotics and was shown to be of value in preventing *Proteus* species swarming. Several other workers[5,6,7] confirmed the non-inhibitory effect upon *N. gonorrhoeae* and its value in preventing swarming.

GC SELECTIVE SUPPLEMENT

Code: SR56

GC Supplement is a sterile lyophilised concentrate of specially prepared yeast fractions, glucose and sodium bicarbonate together with the selective agents VCNT.

Vial contents (each vial is sufficient for 500ml of medium)

Yeast autolysate	5.0g
Glucose	0.75g
Sodium bicarbonate	0.075g
Vancomycin	1.5mg
Colistin sulphate	3.75mg
Nystatin	6,250 IU
Trimethoprim	2.5mg

GC Supplement contains the appropriate growth factors and antibiotics to prepare Thayer Martin Medium[4,7]. This supplement is recommended for those laboratories that prefer to use yeast extract as the source of essential growth factors required by pathogenic Neisseria.

LCAT SELECTIVE SUPPLEMENT

Code: SR95

An antibiotic supplement for the isolation of N. gonorrhoeae and N. meningitidis.

Vial contents (each vial is sufficient for 500ml of medium)

Lincomycin	0.5mg
Colistin	3.0mg
Amphotericin B	0.5mg
Trimethoprim	3.25mg

Young[8] described a modification of the New York City Medium to which the above antibiotics were added. Lincomycin was found to be less inhibitory to gonococcus than vancomycin at 3μg/ml[9,10]. Nystatin was replaced by amphotericin B which was found to be a more active anti-fungal agent than nystatin, suppressing the yeast contaminants often found in vaginal and urethral specimens[11].

MEDIUM		CONSTITUENTS		
		Soluble Haemoglobin or Blood	**Growth Supplement**	**Selective Supplement**
Thayer Martin Medium (non-selective)	Oxoid GC Agar Base CM367	Soluble Haemoglobin L53	Vitox SR90	–
Thayer Martin Medium	Oxoid GC Agar Base CM367	Soluble Haemoglobin L53	Vitox SR90	VCN Antibiotic Supplement SR101
Thayer Martin Medium (modified) with Vitox	Oxoid GC Agar Base CM367	Soluble Haemoglobin L53	Vitox SR90	VCNT Antibiotic Supplement SR91
Thayer Martin Medium (modified) with Yeast Fractions	Oxoid GC Agar Base CM367	Soluble Haemoglobin L53	GC Supplement SR56	GC Supplement SR56
'Transgrow' Medium	Oxoid GC Agar Base CM367 + 1% Agar	Soluble Haemoglobin Supplement SR56	with Vitox SR90 *or* GC Supplement SR56	VCN SR101 *or* VCNT SR91 *or* GC Supplement SR56
GC Medium derived from the New York City formulation	Oxoid GC Agar Base CM367	Lysed Defibrinated horse blood or Laked horse blood SR48	Yeast Autolysate Supplement SR105	LCAT Antibiotic Supplement SR95 *or* VCAT Antibiotic Supplement SR104

VCAT SELECTIVE SUPPLEMENT

Code: SR104

An alternative antibiotic supplement for the isolation of N. gonorrhoeae and N. meningitidis.

Vial contents (each vial is sufficient for 500ml of medium)

Vancomycin	1.0mg
Colistin sulphate	3.75mg
Amphotericin B	0.5mg
Trimethoprim	1.5mg

Faur et al[12] described a medium in which the above antibiotics were added.

Nystatin was replaced with amphotericin B which was found to be a more active anti-fungal agent, suppressing most yeast contaminants. The vancomycin level was reduced from 3µg/ml to 2µg/ml to ensure that the 3% of gonococcus strains shown by Reyn[9] to be sensitive to 3µg/ml of vancomycin were not inhibited[11].

Directions for preparation of the Variant Media

Thayer Martin Medium with GC Supplement

1 Suspend 18g of Oxoid GC Agar Base in 235ml of distilled water and bring to the boil to dissolve the agar. Sterilise by autoclaving at 121°C for 15 minutes.
2 Prepare a 2% solution of Soluble Haemoglobin Powder L53 by adding 250ml of warm distilled water to 5g of haemoglobin powder. Continually stir the solution during the addition of water. Sterilise by autoclaving at 121°C for 15 minutes.
3 Dissolve the contents of a vial of sterile GC Supplement SR56 in 15ml of sterile distilled water.
4 Aseptically add the GC Supplement Solution SR56 to the 235ml of sterile GC Agar Base cooled to 50°C and mix gently. Add the 250ml of sterile haemoglobin solution, cooled to 50°C, to the GC Agar Base-Supplement solution. Mix gently to avoid trapping air bubbles in the agar and pour into sterile petri dishes.

Thayer Martin Medium with Vitox and either VCN or VCNT Antibiotic Supplement.

1 Suspend 18g of GC Agar Base in 240ml of distilled water and bring gently to the boil to dissolve the agar. Sterilise by autoclaving at 121°C for 15 minutes.
2 Prepare a 2% solution of Soluble Haemoglobin Powder L53 by adding 250ml of warm distilled water to 5g of haemoglobin powder. Continually stir the solution during the addition of water. Sterilise by autoclaving at 121°C for 15 minutes.
3 Dissolve the contents of one vial of Vitox SR90 as directed on the vial label.

4 Dissolve the contents of a vial of either VCN Antibiotic Supplement SR101 or VCNT Antibiotic Supplement SR91 as directed on the vial label.
5 Aseptically add the Vitox solution to 240ml of sterile GC Agar Base cooled to 50°C.
6 Aseptically add the reconstituted antibiotic supplement VCN or VCNT to the GC Agar Base-Vitox solution.
7 Asceptically add the 250ml of sterile haemoglobin solution, cooled to 50°C to the GC Agar Base-Vitox-Antibiotic Supplement solution. Mix gently to avoid trapping air bubbles in the agar and pour into sterile petri dishes.

'Transgrow' Medium
'Transgrow' Medium is prepared in the same manner as described for Thayer Martin Medium variants, except that 5g of Agar is added to the 18g of GC Agar Base. The medium is dispensed as agar slants in glass bottles. The extra glucose required is contained in the growth supplements.

GC Medium derived from the New York City Formulation with LCAT or VCAT antibiotic supplement
1 Suspend 18g of Oxoid GC Agar Base in 425ml of distilled water and bring gently to the boil to dissolve the agar. Sterilise by autoclaving at 121°C for 15 minutes.
2 Lyse 50ml of Defibrinated Horse Blood SR50 with 0.5% (by vol.) saponin.
3 Dissolve the contents of a vial of sterile Yeast Autolysate Supplement SR105 in 15ml of sterile distilled water.
4 Dissolve the contents of either LCAT SR95 or VCAT SR104 in 10ml of sterile distilled water.
5 Aseptically add the lysed defibrinated horse blood, Yeast Autolysed Supplement and the Antibiotic Supplement (LCAT or VCAT) to the sterile GC Agar Base cooled to 50°C. Mix gently to avoid trapping air bubbles in the agar and pour into sterile petri dishes.

This is a derivative of NYC Medium[11,12,13] based on Young's publication[14] where the higher level of glucose recommended by the originators was reduced to allow sugar fermentation test to be carried out[15].

Technique
1 Prepare the medium as directed and pour into petri dishes.
2 Inoculate by rolling the culture swabs across a segment of the plate or preferably in a large 'Z' pattern so that an adequate area of the plate is inoculated. Streaking of the plate is carried out with a sterile loop to ensure adequate dispersion of the organisms.
3 Plates are incubated at 37°C in a sealed jar with at least 70% humidity and 5–10% carbon dioxide provided by the introduction of the gas into the incubator or by the use of a candle jar. The above incubation conditions can be conveniently achieved by using the Oxoid Carbon Dioxide Gas Generating Kit BR39 in the Oxoid Gas Jar HP11.
4 Examine the plates after 24 hours incubation and if negative reincubate for a further 24 hours.
5 Presumptive gonococcus colonies are identified by the Gram stain, oxidase and sugar fermentation reactions.

See also Mycoplasma page 2-120.

Typical reactions of *N. gonorrhoeae*
Gram negative cocci, usually arranged in pairs with long axes parallel. Oxidase positive.

Fermentation Reactions of the *Neisseria* Group

	Glucose	Maltose	Lactose	Sucrose
N. meningitidis	+	+	–	–
N. gonorrhoeae	+	–	–	–
Bran. catarrhalis	–	–	–	–
N. flava and related types	+	+/–	–	+/–

+ = Acid production
+/– = Variation in reaction among different types

List of products available from Oxoid for the Growth and Identification of *Neisseria* Species
GC Agar Base CM367
VCN Selective Supplement SR101
Agar No.1 L11
VCNT Selective Supplement SR91
Soluble Haemoglobin Powder L53
LCAT Selective Supplement SR95
Sterile Yeast Autolysate Supplement SR105
VCAT Selective Supplement SR104
Vitox SR90
GC Supplement SR56
(Yeast Fractions plus VCNT Antibiotics)
Defibrinated Horse Blood SR50
Oxidase Detection Papers BR54
Laked Horse Blood SR48

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control
Positive control:
with antibiotics
Neisseria gonorrhoeae ATCC® 19424
Neisseria meningitidis ATCC® 13090

without antibiotics
Haemophilus influenzae ATCC® 35056

Negative control:
with antibiotics
Proteus vulgaris ATCC® 13315
Staphylococcus aureus ATCC® 25923

Please note that for the LCAT Selective Supplement SR95, *Proteus vulgaris* ATCC® 13315 as a negative control is inoculum dependent.

without antibiotics
Uninoculated medium

Precautions
Use Dacron® or calcium alginate swabs for specimen collection, avoid cotton wool.

Any suspected *Neisseria*-containing specimen should be inoculated onto primary isolation media immediately on collection. If this is not possible then *N. gonorrhoeae* swabs are better held at 4°C for not more than 3 hours. *N. meningitidis* swabs should not be chilled and they should be inoculated on to pre-warmed media.

It is a wise precaution to inoculate GC media with and without antibiotics in parallel because some strains of neisseriae may fail to grow in the presence of antibiotics, especially vancomycin[16].

Humidity is essential for the successful isolation of gonococci. If the prepared plates look dry moisten the surface with a few drops of sterile broth and allow it to soak into the agar before inoculation. Do not flood the plate with broth. Place damp gauze or paper towels in the CO_2 container before incubation.

Hosty et al.[17] showed that the usual transport media are not totally reliable for *N. gonorrhoea*. Inoculation of the sample on to the surface of medium slants in bottles is preferable. The incubator temperature should be set at 35°C because many strains of *N. gonorrhoea* will not grow well at 37°C on culture media[18].

It has been shown that agars vary widely in their toxicity for *N. gonorrhoea* and may be a major factor preventing the growth of gonococci on solid media[19].

The Gram morphology, colony characteristics and oxidase tests form the presumptive identification for the genus *Neisseria*. All presumptive neisseriae should be confirmed by carbohydrate fermentation tests and fluorescent antibody or other serological tests[20].

An ONPG test will detect lactose-utilising strains of neisseriae e.g. *N. lactamica*[21].

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GIOLITTI-CANTONI BROTH

Code: CM523

An anaerobic enrichment broth for Staphylococcus aureus.

Formula	gm/litre
Tryptone	10.0
'Lab-Lemco' powder	5.0
Yeast extract	5.0
Lithium chloride	5.0
Mannitol	20.0
Sodium chloride	5.0
Glycine	1.2
Sodium pyruvate	3.0
pH 6.9 $\pm$ 0.2	

Directions
Suspend 54.2 grams in one litre of distilled water and heat gently to dissolve. Dispense 19ml amounts into 20mm x 200mm test tubes and sterilise by autoclaving at 121°C for 15 minutes. Cool rapidly then aseptically add to each tube 0.3ml of a sterile solution of Potassium tellurite 3.5% SR30.

The medium requires the addition of a 3.5% solution of Potassium tellurite when there is a direct addition of 1 gram of the sample to 19mls of broth. This level of Potassium tellurite is necessary to suppress the high numbers of contaminating organisms that could be expected.

The use of a diluted solution of Potassium tellurite is applicable when a 1 in 10 dilution of the food sample is carried out[1]. In such cases the SR30 should first be diluted 1 in 10 with sterile distilled water.

The addition of 0.1% Tween 80 can be recommended in order to improve recovery of heat injured *Staph. aureus* cells e.g. from milk powder. 1 gram of Tween 80 should be added to 1 litre of CM523 prior to autoclaving[2].

Description
Oxoid Giolitti-Cantoni Broth, a tellurite-mannitol-glycine enrichment broth, based on the formulation of Gott and Cantoni[3] is used for the selection and enrichment of *Staphylococcus aureus* from foodstuffs. Mannitol and sodium pyruvate are growth stimulants for staphylococci and aid detection of the organism when present in small numbers only[4].

The growth of Gram-negative lactose fermenting bacilli are inhibited by lithium chloride and Gram-positive bacilli are inhibited by Potassium tellurite in combination with glycine.

The creation of anaerobic conditions by overlaying with 2cm of sterile paraffin wax inhibits the growth of micrococci.

Giolitti-Cantoni Broth is recommended for the detection of *Staphylococcus aureus* in dried baby milk and other weaning foods where the organism should be absent from 1 gram of test material[6].

The medium is suitable for the examination of meat and meat products[7]. For this purpose the concentration of the Potassium tellurite must be reduced to 0.35% and it is recommended that the weight of the test sample should be reduced to 0.1–0.01 gram.

Technique
The medium should be inoculated as soon as it has been cooled after autoclaving. If there is a delay in putting the medium to use it must be freed from dissolved air by immersion in free flowing steam for 20 minutes.

Inoculate 1 gram of sample material and 1ml aliquots of a series of suitable decimal dilutions into tubes containing 19ml of Giolitti-Cantoni Broth. Two tubes are used for the sample material and for each of the dilutions. This increases the likelihood of detecting *Staphylococcus aureus* when it is present in very small numbers.

The medium is overlaid with 2cm of molten sterile paraffin wax (melting temperature 42–44°C) and incubated for 48 hours at 35°C, examining daily. The result is considered negative for *Staphylococcus aureus* if no blackening of the medium is observed. If blackening does occur at the bottom of the tubes or general blackening of the medium, the broth is streaked on to a staphylococcal isolation medium, such as Baird-Parker Medium[8] CM275 and incubated at 35°C for 24–48 hours. The result is considered positive if black colonies, with a narrow white margin, surrounded by a zone of clearing, are seen.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923

Negative control:
Staphylococcus epidermidis ATCC® 12228

References

1 IDF International Standard 60A:1978.
2 Chopin et al (1985) *J. Food Prod. 48 No.1 21–27.*
3 Giolitti C. and Cantoni C. (1966) *J. Appl. Bact. 29. 395.*
4 Baird-Parker A. C. (1962) *J. Appl. Bact. 25. 12.*
5 Lambin S. and German A. (1961) *'Précis de microbiologic' p.63, Paris Masson.*
6 Mossel D. A. A., Harrewijn G. A. and Elzebroek J. M. (1973) *UNICEF.*
7 ISO/DIS 5551 (1177) *Part 2.*
8 De Waart J., Mossel D. A. A., Ten Broeke R. and Van de Moosdijk A. (1968) *J. Appl. Bact. 31. 276.*

HAEMOPHILUS TEST MEDIUM (HTM)

HTM BASE

Code: CM898

HTM SUPPLEMENT

Code: SR158
A medium specifically formulated for the susceptibility testing of Haemophilus influenzae.

HTM BASE (CM898)

Formula	gm/litre
Mueller-Hinton Agar	38.0
Yeast extract (specifically selected for low antagonist levels)	5.0
pH 7.4 ± 0.2	

HTM SUPPLEMENT (SR158)

Vial contents

NAD	7.5mg	(equivalent to 15mg/L)
Haematin	7.5mg	(equivalent to 15mg/L)

Directions
Suspend 21.5g of Haemophilus Test Medium Base (CM898) in 500ml of distilled water. Bring to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of 1 vial of Haemophilus Test Medium Supplement (SR158), reconstituted with 2ml of sterile distilled water. Mix well and pour into sterile petri dishes.

Description
Haemophilus Test Medium (HTM) has been specifically formulated for the susceptibility testing of *Haemophilus influenzae*. The medium forms part of the recommended methods of the United States National Committee for Clinical Laboratory Standards (NCCLS)[1].

Haemophilus influenzae require complex media for growth. These complex media have aggravated the routine susceptibility testing of *H. influenzae* because of antagonism between some essential nutrients and certain antimicrobial agents. Difficulties in interpreting inhibition zones may also arise.

Oxoid Haemophilus Test Medium (HTM) is based on the formulation developed by Jorgensen et al[2] which is now recommended by the United States NCCLS. The results achieved using HTM have been found to be highly reproducible[3,4]. Comparisons with Mueller-Hinton Chocolate Agar have shown an overall agreement of 99.6%[5].

The transparency of the medium allows zones of inhibition to be read easily through the bottom of the petri dish. HTM contains low levels of antimicrobial antagonists, which allows testing of trimethoprim/sulphamethoxazole to be carried out with greater confidence.

Technique
1 Prepare the inoculum in Mueller-Hinton Broth (Oxoid CM405) or 0.9% saline, to match the turbidity of 0.5 McFarland standard.
2 Using a swab saturated with the above inoculum suspension, inoculate the surface of a Haemophilus Test Medium Agar plate to give confluent growth.
3 Apply the antimicrobial discs on to the surface of the Haemophilus Test Medium plate.
4 Incubate the plates at 35°C in 5–7% carbon dioxide for 16–18 hours and measure the zones of inhibition.

NB: In the absence of a CO_2 incubator, the CO_2 enriched atmosphere can best be achieved by using the Oxoid Gas Generating Kit BR39 in conjunction with Oxoid Anaerobic Jar.

Storage conditions and Shelf life
HTM Base should be stored tightly capped in the original container in a cool, dry place away from bright light. When stored as directed the medium will remain stable until the expiry date printed on the label.

HTM Supplement as supplied should be stored in the dark below 0°C. When stored as directed the supplement is stable until the expiry date stated on the label.

Quality Control
H. influenzae ATCC®49766 Good growth

References

1 NCCLS Documents M2-A4 Vol. 10. No. 7. and M7-A2 Vol. 10. No 8.
2 Jorgensen J.H., Redding J.S., Maher L.A. and Howell A.W. (1987) *J. Clin. Micro. 25, 2105–2113.*
3 Doern G.V., Jorgensen J.H., Thornsberry C. and Snapper H. (1990). *Eur. J. Clin. Microbiol. Infect. Dis. 9, 329–336.*
4 Barry A.L., Jorgensen J.H. and Hardy D.J. (1991) *J. Antimic. Chem. 27, 295–301.*
5 Evans G., Marsik F., Thompson L. and Fowler J. (1990) *Abstracts of ASM Meeting 1990 C-252.*

HEKTOEN ENTERIC AGAR

Code: CM419

A differential, selective medium for the isolation of Shigella and Salmonella species from enteric pathological specimens.

Formula	gm/litre
Proteose peptone	12.0
Yeast extract	3.0
Lactose	12.0
Sucrose	12.0
Salicin	2.0
Bile salts No.3	9.0
Sodium chloride	5.0
Sodium thiosulphate	5.0
Ammonium ferric citrate	1.5
Acid fuchsin	0.1
Bromothymol blue	0.065
Agar	14.0
pH 7.5 ± 0.2	

Directions
Suspend 76g of the medium in 1 litre of distilled water and soak for 10 minutes. Heat gently and allow to boil for a few seconds to dissolve the agar. DO NOT AUTOCLAVE. Cool to 50°C and pour plates.

Description
Hektoen Enteric Agar was developed by King & Metzger[1]. The high peptone content offsets the inhibitory effect of bile salts on *Shigella* species in particular. The additional carbohydrates (sucrose and salicin) give better differentiation than lactose alone and the lower toxicity of the double indicator improves recovery. The increased lactose content helps early recognition of slow lactose-fermenting organisms. The thiosulphate and ferric citrate are present to detect H_2S-producing organisms.

Taylor & Schelhaut[2] found the medium to be of value in the differentiation of pathogenic organisms and for better growth of shigellae.

Hoben et al.[3] added novobiocin 15mg/litre to improve the selectivity of the medium by inhibiting *Citrobacter* and *Proteus* species.

Hektoen Enteric Agar meets the requirements of the APHA[4].

Technique
Inoculate the medium with fresh faeces suspended in Ringers solution or inoculate directly with rectal swabs. Spread the inoculum to obtain well separated colonies. Incubate for 18–24 hours at 37°C. Further incubation will improve differentiation between shigellae and salmonellae.

Organism characteristics:

Shigella	Green, moist raised colonies.
Salmonella	Blue-green colonies with or without black centres.
Coliforms (rapid lactose/sucrose/ salicin fermenters)	Salmon-pink to orange colonies surrounded by a zone of bile precipitation.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control
Positive control:
Salmonella typhimurium ATCC® 14028
Shigella flexneri ATCC® 12022

Negative control:
Escherichia coli ATCC® 25922
Enterococcus faecalis ATCC® 29212

Precautions
Do not overheat the medium or hold it molten for long periods.

Proteus species may resemble salmonellae or shigellae. Further testing must be carried out to confirm the presumptive identification of organisms isolated on this medium.

References

1 King S. and Metzger W. I. (1968) *Appl. Microbiol. 16. 577–561.*
2 Taylor W. I. and Schelhaut D. (1971) *Appl. Microbiol. 21. 32–37.*

3 Hoben D. A., Ashton D. H. A. and Peterson A. C. (1973) *Appl. Microbiol. 21. 126–129.*
4 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.*

HELICOBACTER PYLORI ISOLATION

HELICOBACTER PYLORI SELECTIVE SUPPLEMENT (DENT)

Code: SR147

A selective supplement for the isolation of H. pylori from clinical specimens.

Vial contents (each vial is sufficient for 500ml of medium)

Vancomycin	5.0mg
Trimethoprim lactate	2.5mg
Cefsulodin	2.5mg
Amphotericin B	2.5mg

Directions
To one vial add 2ml of sterile distilled water and dissolve the powder completely. Avoid frothing. Add the contents aseptically to 500ml of sterile Columbia Blood Agar Base CM331 at 50°C. Add 35ml of Laked Horse Blood SR48 and mix well before pouring into sterile petri dishes.

Description
Helicobacter pylori is associated with a number of gastric conditions, chiefly gastritis and peptic ulcers[1,2,3].

Helicobacter pylori Selective Supplement (Dent) SR147 was developed from Dent's selective medium described for the isolation of *H. pylori* from gastric biopsies[2]. This is a modification of Skirrow's medium[4] in which polymixin B is replaced by cefsulodin and amphotericin B is added to inhibit *Candida* species.

When used routinely in the laboratory for 100 gastric biopsies, Dent's medium achieved a higher isolation rate for *H. pylori* and lower contamination by other organisms when compared with Skirrow's medium and chocolate blood agar[2]. The provision of a good selective medium for *H. pylori* will help establish the role of this organism in the aetiology of gastric disease.

Technique
1 Prepare the medium as directed. The plates can be stored at 4°C for three weeks but it is essential that they are kept moist. This can be achieved simply by keeping the plates in a plastic bag.

2 Smear the specimen on to the medium.

Note – the recovery of *H. pylori* from gastric biopsies is improved by direct cultivation as soon as possible after collection. If transportation is necessary, then place the biopsy against the neck of a small, sterile glass bottle containing 0.1ml of sterile saline[2]. The biopsy should adhere to the glass but be protected from dehydration by water vapour.

3 Incubate at 35°C for three to five days under microaerophilic conditions. Use Campylobacter Gas Generating Kit BR56 or BR60 with an active catalyst BR42. Alternatively use CampyGen CN025A or CN035A. CampyGen does not require the addition of water or a catalyst.

4 Examine for the presence of discrete, translucent and non-coalescent colonies. Note that colonies of *H. pylori* do not resemble those of *Campylobacter* species.

5 Confirm the identity of the isolates with the following tests:

Gram negative, curved or spiral bacillus.

Growth at 35°C, no growth at 25°C, variable growth at 42°C. Urease positive, Catalase positive, Oxidase positive, Hippurate negative.

Quality Control
Positive control:
Helicobacter pylori NCTC 11916/ATCC® 43526

Negative control:
Candida albicans ATCC® 10231

References
1 Marshall B. K., Warren J. R., Blincow E. D., Phillips M., Goodwin C. S., Murray R., Blackbourne S. J. and Waters T. E. (1988) *The Lancet, December 24/31, No 8626/8627.*
2 Dent J. C. and McNulty C. A. M. (1988) *Eur. J. Clin. Microbiol. Infec. Dis. 7. 555–568.*
3 Buck G. E. (1988) *Laboratory Management, 26, No.9.*
4 Skirrow M. B. (1977) *BMJ, 1. 9–11.*

HOYLE MEDIUM BASE

Code: CM83

A modification of Neill's medium for the isolation and differentiation of Corynebacterium diphtheriae types.

Formula	gm/litre
'Lab-Lemco' powder	10.0
Peptone	10.0
Sodium chloride	5.0
Agar	15.0
pH 7.8 ± 0.2	

Directions
Suspend 40g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 55°C and add 50ml of Laked Horse Blood SR48 and 10ml of 3.5% Potassium Tellurite solution SR30, mix, and pour plates.

Description
Hoyle medium is the well known modification[1] of Neill's medium for the cultural isolation and differentiation of *Corynebacterium diphtheriae* types. Hoyle medium does not exert the inhibitory effect manifested by Neill's on some *mitis* types, but gives very rapid growth with all types of *Corynebacterium diphtheriae*, so that diagnosis is possible after 18 hours' incubation.

Technique
This is a highly selective medium which is used in parallel with non-selective media such as blood agar (e.g. Blood Agar Base CM55 with 10% of horse blood). Unlike the non-selective media, Hoyle medium should be inoculated by rubbing the throat swab (or other material) over the entire surface – spreading with a platinum loop is not necessary.

Normally incubation for 18 hours at 35°C is sufficient but, when a negative result is obtained, incubation for up to 72 hours may be advisable. Gentian violet stained films made from colonies picked straight off the medium, are satisfactory for *C. diphtheriae* morphology. For the demonstration of the characteristic morphology and staining reactions of *C. diphtheriae* by Neisser's or Albert's method, it is preferable to utilise colonies from Loeffler medium. The toxigenicity of *C. diphtheriae* strains may be determined by the Elek[2] method.

Colonial Characteristics
It is best to examine with a low-power microscope, the colonies being illuminated from above by daylight.

Type differentiation is good, and typical colonial appearances after 18 hours' incubation are as follows:

C. diphtheriae type *gravis* – grey colonies, 1.5–2.5mm diameter dull, matt surface. May be slightly umbonate and show indented margins. Colonies can be pushed along the surface of the medium.

C. diphtheriae type *mitis* -- grey colonies, 1.5–2.0mm diameter with regular margins and shining surface. Variation in size common.

C. diphtheriae type *intermedius* – grey colonies, 0.5–0.75mm diameter with shining surface. Colonies are very uniform in size with darker centres.

C. hofmannii – usually rounded, white or greyish, 0.5–0.75mm diameter. Colonies may be up to 1mm diameter, when they are black in more heavily inoculated areas and white when well isolated.

C. xerosis – black shining colonies of variable size.

Streptococci – minute black or brownish-black colonies.

Other organisms may occasionally grow which resemble *C. diphtheriae* type *intermedius* but are larger, while sporing anaerobes may produce brownish mucoid colonies.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control
Positive control:
Corynebacterium diphtheriae biotype *gravis* ATCC® 19409
Corynebacterium diphtheriae biotype *intermedius* ATCC® 14779

Negative control:
Uninoculated medium.

Precautions
It should be noted that not all corynebacteria produce the typical colonies described above – so in all cases it is advisable to use Hoyle medium in conjunction with the additional media and tests mentioned above.

References
1 Hoyle L. (1941) *Lancet. i. 175–176.*
2 Elek S. D. (1948) *Brit. Med. J. 1. 493–496.*

HIGH RESOLUTION (H.R.) MEDIUM

Code: CM845

A chemically defined medium for fluconazole susceptibility testing.

Formula	gm/litre
Dextrose	19.98
Potassium dihydrogen phosphate	1.99
Ammonium sulphate	4.99
L-Glutamine	0.58
Magnesium sulphate (anhydrous)	0.99
Sodium chloride	0.20
Calcium chloride	0.20
L-Lysine monohydrochloride	0.073
Valine	0.047
L-Arginine monohydrochloride	0.042
L-Histidine	0.023
Methionine (DL)	0.0189
Tryptophane	0.020
Inositol	0.00397
Boric acid	0.00099
Calcium D-pantothenic acid	0.00079
Nicotinic acid	0.00079
Pyridoxine hydrochloride	0.00079
Aneurine hydrochloride	0.00079
Manganous sulphate	0.00079
Zinc sulphate	0.0014
4-Aminobenzoic acid	0.000395
Riboflavin BP/USP	0.000395
Ferric chloride	0.000395
Cupric sulphate	0.00012
Biotin crystalline	0.000004
Folic acid	0.000395
L-Isoleucine	0.052
Sodium molybdate	0.00047
Potassium iodide	0.00020
L-Leucine	0.052
Threonine	0.0476

Directions
PART A
Prepare a 2% w/v solution of Agar Technical (Oxoid L13) and buffer to pH 7.5 with 0.1ml phosphate buffer. Sterilise at 115°C for 10 minutes.

PART B
Dissolve 29.34g of High Resolution Medium in 900ml of distilled water. Stir continuously and add 2g of sodium bicarbonate (analar) and make up the volume to 1 litre with distilled water. Sterilise by filtration. The medium can be kept at 4°C for 2 weeks.

Aseptically mix equal volumes of molten High Resolution Medium Part A (cooled to 60°C) and High

Resolution Medium Part B. Mix thoroughly and pour into sterile petri dishes.

Description
High Resolution Medium is a chemically defined medium, specifically developed for the *in vitro* testing of fluconazole. The MIC values generated using the medium give sensible correlations with efficacy *in vitro* and with clinical outcome.

In a comparison of a disc diffusion method against a microdilution method correlation using HR medium was good for a number of antibiotics including nystatin and amphotericin B but generally better for new triazoles such as fluconazole than for the other antifungals.[6]

Technique
Preparation of the inocula
The preparation and standardisation of the inoculum varies with different fungi:

CANDIDA spp
1 Grow *Candida* spp overnight at 37°C in Sabouraud dextrose broth.
2 Vortex mix and make a 1 in 100 dilution of each culture in normal saline and estimate the cell numbers using a haemocytometer.
3 Appropriately dilute each culture in normal saline to give the following cell densities:

Candida albicans	10^5/ml
Candida krusei	10^5/ml
Candida tropicalis	10^5/ml
Candida guillermondii	10^6/ml
Candida parapsilosis	10^6/ml
Candida pseudotropicalis	10^6/ml

Inoculation of the plates
1 Surface inoculate each series of plates using a multipoint inoculator which delivers 1µl of each culture inoculum. It is possible to inoculate 36 isolates per 9cm petri dish. Inoculate control plates at both the beginning and end of the inoculation run.
2 Incubate the plates at 28°C for 48 hours.

Endpoint determination
1 Check the control plates to ensure that all the organisms have grown adequately.
2 Read all plates against a standard background and record, for each isolate, the lowest concentration of fluconazole that completely suppresses visible (to the naked eye) growth. This is the MIC value.

DERMATOPHYTE spp
1 Grow the dermatophytes on Sabouraud Dextrose Agar (CM41) at 28°C for 5–10 days.
2 Scrape off the mycelium from the agar surface using a scalpel and place in a bijou bottle containing 4gm of glass beads (approximately 2mm diameter) plus 2ml of 0.85% saline.
3 Vortex mix to evenly disperse.
4 Adjust the density of the suspension with 0.85% saline to give a 65% light transmission on an absorptiometer.
5 Inoculate the plates using a multipoint inoculator. It is possible to inoculate 20 isolates, evenly spaced, per 9cm petri dish.
6 Incubate at 28°C for 6 days.
7 Check the control plates to ensure that all isolates have grown adequately.
8 Determine the endpoint as for *Candida*.

Candida albicans (ATCC® 76615) has an MIC value of 1.56µg/ml against the antifungal agent fluconazole.

Storage conditions and Shelf life
High Resolution Medium should be stored tightly capped in the original container at 10 to 25°C away from bright light. When stored as directed the medium will remain stable until the expiry date printed on the label.

Please note shelf life of 2 years.

References

1 Hoeprich P.D. and Finn P.D. (1972) *J. Infect. Dis. 126, 353–361.*
2 Cook R.A., McIntyre K.A. and Galgiani J.N. (1990). *Antimicrob. Agents and Chemother. 34, 1542–1545.*
3 Pfaller M.A. et al. *Antimicrob. Agents Chemother. 34, 1648–1654.*
4 Pfaller M.A. et al. (1992) *Antimicrob. Agents and Chemother. 36, 1805–1809.*
5 Pfizer Ltd, private communication (1990).
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IRON SULPHITE AGAR

Code: CM79

A medium for the detection of thermophilic anaerobic organisms.

Formula	gm/litre
Tryptone	10.0
Sodium sulphite	0.5
Iron (III) citrate	0.5
Agar	12.0
pH 7.1 ± 0.2	

Directions
Suspend 23.0g in 1 litre of distilled water and boil to dissolve completely. Sterilise by autoclaving for 15 minutes at 121°C. Mix well before pouring.

Description
This medium is a modification of Cameron Sulphite Agar developed by the National Canners Association of America[1].

It had been shown that the medium was improved by reducing the concentration of sodium sulphite. Beerens[2] showed that some strains of *Clostridium sporogenes* would not tolerate 0.1% sulphite. This was confirmed by Mossel et al.[3] who consequently used iron sulphite agar containing only 0.05% sulphite and obtained no apparent inhibition.

Technique
Iron Sulphite Agar is particularly suitable for the detection of thermophilic anaerobic organisms causing sulphide spoilage in food. The medium

should be dispensed in 10ml amounts in tubes for deepshake cultures, and inoculated whilst fluid at about 50°C. Allow to set and incubate at 55°C for thermophilic species. *Desulfotomaculum nigrificans*, the type species, produces distinct black spherical growths in the depth of the medium.

In the Attenborough and Scarr method[4], diluted samples of the sugar were filtered through membrane filters which were then rolled up and placed in tubes containing enough melted Iron Sulphite Agar (at approximately 50°C) to cover them. The medium was allowed to solidify and the tubes were incubated at 56°C. After 48 hours the number of black colonies on the membrane was counted. This membrane filter technique is quicker than the standard method but of comparable accuracy, and permits the examination of a much larger sample[5].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium below 25°C.

Quality Control

Positive control:
(blackening)
Clostridium sporogenes ATCC® 19404
Desulfotomaculum nigrificans ATCC® 19858

Negative control:
(no blackening)
Escherichia coli ATCC® 25922

Precautions
The blackening reaction is only presumptive evidence of clostridial growth. Confirmation tests must be carried out to identify the organism growing in the medium.

References

1 Tanner F. W. (1944) *'The Microbiology of Foods' 2nd ed., Garrard Press, Illinois p. 1127.*
2 Beerens H. (1958) *DSIR, Proc. 2nd Internat. Symp. Food Microbiol. 1957, HMSO, London, pp. 235–245.*
3 Mossel D. A. A., Golstein Brouwers G. W. M. V. and de Bruin A. S. (1959) *J. Path. Bact. 78. 290–291.*
4 Attenborough Sheila J. and Scarr M. Pamela (1957) *J. Appl. Bact. 20. 460–466.*
5 Bufton A. W. J. (1959) *J. Appl. Bact. 22. 278–280.*

'ISO-SENSITEST AGAR'

Code: CM471

A closely defined medium with stabilised mineral content for antimicrobial susceptibility testing.

Formula	gm/litre
Hydrolysed casein	11.0
Peptones	3.0
Glucose	2.0
Sodium chloride	3.0
Soluble starch	1.0
Disodium hydrogen phosphate	2.0
Sodium acetate	1.0
Magnesium glycerophosphate	0.2
Calcium gluconate	0.1
Cobaltous sulphate	0.001
Cupric sulphate	0.001
Zinc sulphate	0.001
Ferrous sulphate	0.001
Manganous chloride	0.002
Menadione	0.001
Cyanocobalamin	0.001
L-Cysteine hydrochloride	0.02
L-Tryptophan	0.02
Pyridoxine	0.003
Pantothenate	0.003
Nicotinamide	0.003
Biotin	0.0003
Thiamine	0.00004
Adenine	0.01
Guanine	0.01
Xanthine	0.01
Uracil	0.01
Agar	8.0
pH 7.4 $\pm$ 0.2	

Directions
Suspend 31.4g in 1 litre of distilled water and bring to the boil to dissolve the agar. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Oxoid 'Iso-Sensitest Agar' was developed specifically for antimicrobial susceptibility tests. Its formulation was carefully constructed to give a reproducible, semi-defined medium in which the undefined components were kept to a minimal level. However, it allows the growth of the great majority of micro-organisms without further supplementation.

It has been designed to overcome the objections made about Mueller-Hinton media by various workers[1,2,3,4,5,6,7].

These objections were:

1 Different MIC values in the broth and agar versions of the medium.
2 Agar versions showing antagonistic effects towards tetracycline.
3 High levels of sulphonamide and trimethoprim antagonists.
4 Poor reproducibility with different manufacturers' peptones.

5 Poor growth-supporting ability for streptococci and variable growth rates with gram-positive organisms generally.

Some mutant strains which are totally dependent on thymine and thymidine for their growth will, however, not grow in Oxoid 'Iso-Sensitest Agar', which has these two compounds at very low levels, as they are the naturally occurring antagonists of trimethoprim. Care must be taken to recognise these strains[8,9,10].

Oxoid 'Iso-Sensitest Agar', CM471 was developed from Oxoid 'Sensitest' Agar CM409 which has been used successfully in several centres throughout the world[11,12,13].

The role of metal ions as antagonists to certain antibiotics has been described by many workers[14,15,16,17,18,19]. A stabilised mineral content in sensitivity test media is, therefore, important.

Reller, Schoenknecht, Kenny & Sherris[3] demonstrated the contribution of cations provided in media by ordinary agars. A considerable difference in mineral content between the agar and broth media was shown. Only by using an agar, which is specially processed to remove the free anions and cations, can a stabilised mineral content be obtained.

Oxoid 'Iso-Sensitest Agar' has stability in mineral content and the ability to produce optimum antimicrobial zones of inhibition.

The amino-nitrogen base of acid-hydrolysed casein and special peptones has been supplemented with defined growth factors. Careful preparation of the nutrients ensures that trimethoprim and sulphonamide antagonists are at very low levels.

THE ADDITION OF LYSED HORSE ERYTHROCYTES TO THE MEDIUM IS NOT REQUIRED WHEN CARRYING OUT ANTIMICROBIAL SUSCEPTIBILITY TESTS WITH THESE COMPOUNDS.

SUPPLEMENTAL NUTRIENTS FOR NUTRITIONALLY DEPENDENT ORGANISMS

Some pathogenic organisms may be nutritionally dependent because of intrinsic demands for special growth factors, e.g. streptococci, neisseria, haemophilus, campylobacter etc. Other organisms may exhibit mutant characteristics, e.g. dwarf *Staph. aureus*, thymidine-dependent *Esch. coli.*

Supplemental nutrients can be added to 'Iso-Sensitest Agar' to obtain or improve growth of these organisms[20].

Nutrient	**Micro-organism**
Laked Blood (5% v/v)	*Neisseria, Streptococcus*
Fildes Peptic Digest of Blood (5% v/v)	*Haemophilus*
Menadione (0.5µg/ml) Thiamine (2µg/ml)	Dwarf colonies of *Staph. aureus and coliform* organisms
Pyridoxine hydrochloride (1µg/ml)	Symbiotic streptococci

Certain supplements interfere with antimicrobial activity and tests must be made to measure their effect.

Nutrient	**Antimicrobial**
Thymidine	*Trimethoprim*
Blood	*Sulphonamides, trimethoprim, aminoglycosides*
CO_2	*Aminoglycosides, erythromycin, lincomycin, tetracyline, novobiocin*
Cysteine and other -SH compounds	*Aminoglycosides*
Vitox/Isovitalex	*Aminoglycosides, sulphonamides, trimethoprim*

'Iso-Sensitest Agar' with the addition of 10% of horse blood has been recommended as a suitable medium when testing susceptibility of *Helicobacter pylori*[21]. Although Mueller-Hinton agar performed nearly as well, 'Iso-Sensitest Agar' was preferred because its contents are better defined.

References

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'ISO-SENSITEST BROTH'

Code: CM473

Formula	gm/litre
Hydrolysed casein	11.0
Peptones	3.0
Glucose	2.0
Sodium chloride	3.0
Soluble starch	1.0
Disodium hydrogen phosphate	2.0
Sodium acetate	1.0
Magnesium glycerophosphate	0.2
Calcium gluconate	0.1
Cobaltous sulphate	0.001
Cupric sulphate	0.001
Zinc sulphate	0.001
Ferrous sulphate	0.001
Manganous chloride	0.002
Menadione	0.001
Cyanocobalamin	0.001
L-Cysteine hydrochloride	0.02
L-Tryptophan	0.02
Pyridoxine	0.003
Pantothenate	0.003
Nicotinamide	0.003
Biotin	0.0003
Thiamine	0.00004
Adenine	0.01
Guanine	0.01
Xanthine	0.01
Uracil	0.01
pH 7.4 $\pm$ 0.2	

Directions
Add 23.4g to 1 litre of distilled water. Mix well and distribute into tubes or bottles. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Oxoid 'Iso-Sensitest Broth' has been produced in parallel with 'Iso-Sensitest Agar'. The broth has an identical nutrient formulation without the specially purified agar. Where studies on antimicrobial susceptibilities are to be made in both broth and agar, this will be found to be of particular value.

Details of the function of the medium and the methodology used for antimicrobial susceptibility tests are discussed in the Section 'Susceptibility Testing'.

Quality Control
Positive control:
Streptococcus pneumoniae ATCC® 6303

(with blood)
Neisseria meningitidis ATCC® 13090

(without blood)
Staphylococcus aureus ATCC® 25923
Enterococcus faecalis ATCC® 29212
Pseudomonas aeruginosa ATCC® 27853

Negative control:
Uninoculated medium

Precautions
Note the comments made in Description about the inhibition of thymine/thymidine dependent organisms.

KANAMYCIN AESCULIN AZIDE AGAR BASE

Code: CM591

A selective medium when used with Kanamycin Supplement SR92 for the isolation of enterococci in foodstuffs.

Formula	gm/litre
Tryptone	20.0
Yeast extract	5.0
Sodium chloride	5.0
Sodium citrate	1.0
Aesculin	1.0
Ferric ammonium citrate	0.5
Sodium azide	0.15
Agar	10.0
Final pH 7.0 $\pm$ 0.2	

Directions
Suspend 21.3g in 500ml of distilled water. Add one vial of Kanamycin Supplement SR92 reconstituted as directed. Bring to the boil. Dispense into final containers and sterilise by autoclaving at 121°C for 15 minutes.

KANAMYCIN SULPHATE SELECTIVE SUPPLEMENT

Code: SR92

Vial contents (each vial is sufficient for 500ml of medium)

Kanamycin sulphate	10mg

Directions
Add 2ml of sterile distilled water to one vial and mix gently to dissolve completely. Add the contents to 500ml of Kanamycin Aesculin Azide Agar Base CM591.

Description
Kanamycin Aesculin Azide Agar Base CM591 replaces KAA Agar CM481. This change has been made to follow the Oxoid Health & Safety rules that antibiotics should not be present in powdered culture media where they can be inhaled or contaminate surfaces.

Kanamycin sulphate is added separately to 500ml of reconstituted agar from freeze-dried vials (Kanamycin Supplement SR92) containing the precise amount of antibiotic required.

The medium contains the selective inhibitory components kanamycin sulphate and sodium azide. It also contains an indicator system to detect the growth of aesculin-hydrolysing streptococci. These organisms produce black zones around the colonies from the formation of black iron phenolic compounds derived from aesculin-hydrolysis products and ferrous iron.

Kanamycin Aesculin Azide Agar was designed by Mossel et al.[1,2] to detect enterococci in foodstuffs.

Round, white or grey colonies about 2mm in diameter, surrounded by black zones of at least 1cm diameter are considered to be enterococci (presumptive).

Incubation is carried out aerobically at 35°C or 42°C 0.3°C for 18–24 hours. The higher incubation temperature increases the selectivity of the medium.

This medium was used by Mossel et al.[3] in the Dip Slide technique for bacteriological monitoring of foods.

Kanamycin-Aesculin-Azide Agar has been used successfully for the isolation of glycopeptide-resistant enterococci from clinical specimens and foods[4,5].

Technique
Inoculation method for samples: spread 0.1ml of sample dilutions over the whole of a pre-dried 9cm diameter plate.

The following procedure for testing foodstuffs is adapted from Mossel, Harrewijn and Elzebroek[4].

1 Prepare tubes of sterilised Tryptone Water CM87 in 9ml volumes. Chill to 0–5°C by storing in a refrigerator for 18 hours prior to use.
2 Add 1g or 1ml of the thoroughly mixed food sample to a tube containing 9ml of pre-chilled diluent (10^{-1} dilution). Shake well for 30 seconds. Sample 1ml of the contents, within 30 seconds after mixing, into a fresh tube of diluent. Continue the process using fresh sterile pipettes until a dilution is reached which will produce 100 colonies per 1ml. Store the decimal dilutions in the refrigerator and examine within 3 hours of their preparation.
3 Streak onto plates of Kanamycin Aesculin Azide Agar CM591 and incubate for 16–24 hours at 35°C ± 1°C. Consider the result positive for enterococci when colonies surrounded by black haloes are grown.
4 Confirmatory tests may be carried out, e.g. catalase test, utilisation of glucose, growth at 45°C ± 1°C, chain-forming Gram-positive cocci.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared media at 2–8°C.

Quality Control
Positive control:
Enterococcus faecium ATCC® 19434
Enterococcus bovis ATCC® 27960

Negative control:
Bacillus subtilis ATCC® 6633
Escherichia coli ATCC® 25922

Precautions
Observe the hazard precautions stated on page 2-7 about sodium azide when disposing the medium. There is no universal medium which will isolate all strains of enterococci[7].

Unless a presumptive count is acceptable all isolates should have their identity confirmed with further tests.

References

1 Mossel D. A. A., Bijker P. G. H. and Eelderink I. (1978) *Arch. Lebensmittel-hyg. 29. 121–127.*
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KF STREPTOCOCCUS AGAR

Code: CM701

A selective medium for the isolation and enumeration of enterococci.

Formula	gm/litre
Proteose peptone	10.0
Yeast extract	10.0
Sodium chloride	5.0
Sodium glycerophosphate	10.0
Maltose	20.0
Lactose	1.0
Sodium azide	0.4
Bromo-cresol purple	0.015
Agar	20.0
Final pH 7.2 ± 0.2	

Directions
Suspend 76.4g in 1 litre of distilled water. Bring to the boil with frequent agitation. Boil for 5 minutes.* Cool to 50°C and add aseptically 1ml of sterile aqueous 1% solution of 2,3,5-Triphenyltetrazolium chloride to each 100ml of medium. Pour into sterile petri dishes when using the membrane filtration method or hold at 45°C when using the pour-plate method.

*Note: The medium can be autoclaved at 121°C for 10 minutes if total selectivity is required.

Description
KF Streptococcus Agar is based on the formulation described by Kenner et al.[1] and is recommended[2] for the detection and enumeration of enterococci in faeces, milk, water and other materials by the pour-plate or membrane filtration methods. The presence of enterococci in the material under test is indicative of faecal pollution by man or animals.

KF Streptococcus Agar Medium is selective for the following Group D and Group Q species.

Enterococcus Group

E. faecalis	Group D
E. faecalis subsp. *liquefaciens*	Group D
E. faecalis subsp. *zymogenes*	Group D
E. faecium	Group D
E. bovis	Group D
E. equinus	Group D
S. avium	Group Q

Streptococcus avium (Group Q) has been included in the 'enterococci' group as it has very similar biochemical and antigenic characteristics to the Group D species and also occurs in warm-blooded animals.

The detection of enterococci may provide more specific information about the source of pollution because certain enterococci are host specific. For example, a predominance of *E. bovis* and *E. equinus* would indicate pollution due to animal excrement.

The detection of *E. bovis* and *E. equinus* species has been found to be associated with pollution involving meat processing plants, dairy wastes and feedlot and farmland run-off.

The detection of these enterococcal species is indicative of recent contamination as the organisms survive for only a short period outside their natural habitat. The coliform/enterococci ratios may also provide information on possible sources of pollution[2].

Caution must be observed when assessing the quality of marine recreational waters, particularly in tropical climates, because a high incidence of false-positive presumptive counts for enterococci may occur. Anaerobic incubation of tropical marine water samples is recommended[3].

Colonies of enterococci on the membrane filter or agar plate are red or pink with a variation in diameter from 0.3 to 2mm. It is recommended that counting should be done with the aid of a low power (10 to 15 magnification) binocular wide field dissecting microscope or equivalent optical device.

Technique

Membrane Filtration Technique

1 Prepare the KF Streptococcus Agar Medium as directed.
2 Filter samples through a sterile membrane to give 20 to 100 colonies on the membrane surface. Use volumes of 100, 10, 1, 0.1 or 0.01ml, depending on the degree of pollution present.
3 Transfer the membrane filter directly to agar medium in the petri dishes, avoiding the formation of air bubbles.
4 Invert the plates and incubate at 35°C for 48 hours.
5 Count all red or pink colonies with the aid of a low power (10 to 15 magnification) binocular wide field dissecting microscope.
6 Calculate the number of enterococci and report as faecal streptococci per 100ml.
7 Confirm the colonies as enterococci.

Plate Count Method

1 Prepare the KF Streptococcus Agar Medium as directed.
2 Prepare dilutions to give a count of 30 to 300 colonies. For most potable water samples plates suitable for counting will be obtained by inoculating 1ml and 0.1ml of undiluted sample and 1ml of sample diluted 1:100m.
3 Measure the selected volume of sample into a petri dish.
4 Pour 15ml of liquified medium into each plate.
5 Thoroughly mix the medium and sample to give a uniform dispersion of the organisms.
6 Solidify the agar as rapidly as possible after pouring.
7 Incubate the plates in an inverted position at 35°C for 48 hours.
8 Count all red to pink surface and subsurface colonies.
9 Calculate the numbers of enterococci and report as faecal streptococci per 100ml.
10 Confirm the colonies as enterococci[2]. Use an inoculating wire to stab through the agar to reach the colonies.

Storage conditions and Shelf life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control

Positive control:
Enterococcus faecalis ATCC® 29212

Negative control:
Escherichia coli ATCC® 25922

Precautions

Observe the HAZARD precautions stated on page 2-7 about sodium azide when disposing of the medium. The pH of the medium should not fall below 7.0 as it may become inhibitory towards enterococci[1]. KF Streptococcus Agar is not specific for the presumptive identification of Group D streptococci. Further tests must be made to confirm the identity of the organisms isolated.

References

1 Kennor G.A., Clark H.F. and Kabler P.W. (1961) *J. Appl. Microbiol. 9. 15–20.*
2 American Public Health Association (1981) *Standard Methods for the Examination of Water and Wastewater, 15th Edn. APHA Inc. Washington DC.*
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KLIGLER IRON AGAR

Code: CM33

A medium for the identification of Enterobacteriaceae, based on double sugar fermentation and hydrogen sulphide production.

Formula	gm/litre
'Lab-Lemco' powder	3.0
Yeast extract	3.0
Peptone	20.0
Sodium chloride	5.0
Lactose	10.0
Glucose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.3
Phenol red	0.05
Agar	12.0
pH 7.4 ± 0.2	

Directions
Suspend 55g in 1 litre of distilled water. Bring to the boil to dissolve completely. Mix well and distribute into containers. Sterilise by autoclaving at 121°C for 15 minutes. Allow to set as slopes with 1 in. butts.

Description
A differential medium for the identification of Enterobacteriaceae on a basis of double sugar fermentation and hydrogen sulphide production. Oxoid Kligler Iron Agar based on the original medium[1,2,3] combines the principles of Russell[4], double sugar agar, with ferric citrate as an indicator to detect hydrogen sulphide production. The medium is recommended for the identification of colonies picked off from plating media such as MacConkey Agar, Bismuth Sulphite Agar, or Desoxycholate Citrate Agar, etc.

Technique
Smear the surface of a Kligler Iron Agar slope and stab the butt with a colony picked off one of the solid media.

There are three reactions to record when interpreting a KIA tube.

1 Carbohydrate utilisation:
(i) slant reaction — *acidity:* yellow colour; *alkalinity:* red colour
(ii) butt reaction — *acidity:* yellow colour; *alkalinity:* red colour

2 Gas production:
aerogenic, bubbles or splitting of agar
anaerogenic, no gas production

3 H_2S production:
blackening in whole or part of butt

Record the slant reaction/the butt reaction/gas production/H_2S production; in that order.

Red slant/yellow butt – glucose only fermented

Yellow slant/yellow butt – glucose + lactose fermented

Red slant/red butt – neither glucose nor lactose fermented.

Reactions after 18–24 hours at 35°C.

Organism	Slope	Butt	Gas	H_2S
Shigella sonnei	Red	Yellow	–	–
Shigella dysenteriae	Red	Yellow	–	–
Salmonella typhi	Red	Yellow	–	+
Salmonella species	Red	Yellow	+	+
Enterobacter species	Red	Yellow	+	–
Klebsiella species	Yellow	Yellow	+	–
Escherichia coli	Yellow	Yellow	+	–
Proteus mirabilis	Red	Yellow	–	+
Morganella species	Red	Yellow	V	–
Citrobacter freundii	Yellow	Yellow	+	+
Yersinia species	Red	Yellow	V	–

V = variable, + = positive, – = negative.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label

Store the prepared medium at 2–8°C.

Quality Control
Positive Control:
Citrobacter freundii ATCC® 8090:
yellow/yellow/+/+.
Proteus vulgaris ATCC® 13315:
red/yellow/–/+.
Alcaligenes faecalis ATCC® 19018:
red/red/–/–.

Negative control:
Uninoculated medium.

Precautions
It is essential that Kligler Iron Agar is examined and reported at 18–24 hours. Early or late readings will give false results.

KIA will grow both oxidative and fermentative organisms. Confusion will result if care is not taken to distinguish between the two groups.

Always use a straight wire to inoculate the butt, to avoid splitting the agar with a loop.

Pure cultures are essential to avoid misinterpretation.

Do not use screw-capped tubes or bottles for KIA medium. It is essential that air is freely available to growth on the slant.

References
1 Kligler I. J. (1917) *Am. J. Pub. Hlth 7. 1042–1044.*
2 Kligler I. J. (1918) *J. Exper. Med. 28. 319–322.*
3 Bailey Sadie F. and Lacey G. R. (1927) *J. Bact. 13. 182–189.*
4 Russell F. F. (1911) *J. Med. Res. 25. 217–229.*

'LAB-LEMCO' AGAR

Code: CM17

A nutrient agar for general bacteriological use, it may be used as the basis for selective, differential, or enriched media.

Formula	gm/litre
'Lab-Lemco' powder	3.0
Peptone	5.0
Agar	15.0
pH 7.4 ± 0.2	

Directions
Suspend 23g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
'Lab-Lemco' Agar is a general purpose medium used for the examination of water and dairy products[1,2].

It is used as a nutrient meat extract agar for general bacteriology and for the preservation of stock cultures. The absence of sodium chloride in the formulation prevents *Proteus mirabilis* forming 'swarming' colonies.

When used as a basal medium, other substances can be added to produce selective, differential or enriched media.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923
Escherichia coli ATCC® 25922

Negative control:
Uninoculated medium

Precautions
'Lab-Lemco' Agar is slightly more alkaline than the medium used in the APHA publications (pH 6.8).

If blood is to be added to the agar then it is necessary to add sodium chloride (0.5% w/v) to prevent haemolysis.

References

1 American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington DC.*
2 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.*

'LAB-LEMCO' BROTH

Code: CM15

A nutrient broth for general bacteriological use, and for the examination of water, sewage, and dairy products by American standard methods.

Formula	gm/litre
'Lab-Lemco' powder	3.0
Peptone	5.0
pH 7.4 ± 0.2	

Directions
Dissolve 8g in 1 litre of distilled water and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
'Lab-Lemco' Broth is a general purpose liquid medium used for the examination of water and dairy products[1,2].

It is used as a nutrient meat extract broth for general bacteriology.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at room temperature (18–22°C).

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923
Escherichia coli ATCC® 25922

Negative control:
Uninoculated medium.

Precautions
'Lab-Lemco' Broth is slightly more alkaline than the medium used in the APHA publications (pH 6.8).

References

1 American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington DC.*
2 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.*

LACTOSE BROTH

Code: CM137

A liquid medium for use in the performance or confirmation of the Presumptive Test for coliforms in water, milk, etc. as specified by the American Public Health Association.

Formula	gm/litre
'Lab-Lemco' powder	3.0
Peptone	5.0
Lactose	5.0
pH 6.9 ± 0.2	

Directions
Dissolve 13g in 1 litre of distilled water and distribute into containers with fermentation tubes (Durham). Sterilise by autoclaving at 121°C for 15 minutes.

Description
Lactose broth is recommended for use in the presumptive identification of coliform organisms in milk, water and foods as specified by the American Public Health Association[1,2,3].

Tubes of Lactose Broth are inoculated with dilutions of the samples and incubated at 35°C. Examination for gas formation is carried out after 24 and 48 hours incubation. This presumptive evidence of coliform organisms must be confirmed by further tests.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at room temperature (18–22°C).

Quality Control
Positive control:
Escherichia coli ATCC® 25922
Enterobacter aerogenes ATCC® 13048

Negative control:
Uninoculated medium

Precautions
Ensure that the fermentation tubes are free from air bubbles before inoculation.

Large water samples may require double-strength lactose broth to reduce the final volumes. Do not overheat double-strength broth or inhibitory products will be produced.

References

1 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Washington DC.*
2 American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington DC.*
3 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.*

LAURYL TRYPTOSE BROTH

(LAURYL SULPHATE BROTH)

Code: CM451

A medium for the detection of coliform organisms in water and waste water, according to the formula of the American Public Health Association.

Formula	gm/litre
Tryptose	20.0
Lactose	5.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	2.75
Potassium dihydrogen phosphate	2.75
Sodium lauryl sulphate	0.1
pH 6.8 ± 0.2	

Directions
Dissolve 35.6g in 1 litre of distilled water and distribute into containers with fermentation tubes (Durham). Sterilise by autoclaving at 121°C for 15 minutes.

Description
Lauryl Tryptose Broth provides a selective medium which is used for the detection of coliform organisms in water, dairy products and other foods. The APHA[1] recommend that Lauryl Tryptose Broth should be used for the Mean Probable Number Presumptive Test of coliforms in waters, effluent or sewage as a confirmatory test of lactose fermentation with gas production for milk samples (APHA[2]) and for the detection of coliforms in foods (APHA[3]).

Surface active agents have long been used as the inhibitory ingredients in selective media. MacConkey[4] introduced bile salts for this purpose and later Albus and Holm[3] working with lactobacilli found that sodium ricinoleate exerted a selective action. The development of synthetic wetting agents opened up new fields of investigation. Mallmann and Darby[6] after comparative tests with a large number of these compounds, showed that sodium lauryl sulphate gave the best results in selective media for the coliform group.

Lauryl Tryptose Broth was designed to promote a rich growth and copious gas production from small inocula of coliform organisms. Aerobic sporing bacteria are completely inhibited. The advantage in using this product is that in addition to giving the fermentation reaction typical of MacConkey Broth it can also be directly tested for the presence of indole. Unlike MacConkey Broth, the medium contains no indicator, but this can be added (if required) after incubation.

Technique
For details of the APHA standard methods please consult the references below.

Lauryl Tryptose Broth is recommended for the detection and enumeration of coliform organisms in water and milk products, especially in the control of ice-cream manufacture and in dairy hygiene. A suggested procedure (Dyett[7]) is as follows:

Inoculate samples of ice-cream into tubes of Lauryl Tryptose Broth in the manner normally employed in the MacConkey test. Examine the tubes after overnight incubation at 35°C and, if no gas is visible, examine again at the end of 48 hours' incubation. For every tube showing fermentation ('primary fermentation') two further tubes of Lauryl Tryptose Broth are inoculated from a tube of the primary fermenting broth, and these are incubated at 35°C and 44°C respectively. It is advisable that the tube to be incubated at 44°C be warmed up in a water bath at this temperature before inoculation.

If the 44°C incubated broth ferments after seven hours, test for indole production with either Ehrlich or Kovac's reagent. Due to the lauryl sulphate present, shaking the reagent culture mixture forms a persistent emulsion which interferes with the test. This may be avoided by shaking with ether, which separates rapidly, and then adding Kovac's reagent to the layer without shaking. If fermentation has not occurred after seven hours, leave the tube overnight at 44°C and test the following day. A positive indole reaction in a broth that has produced gas at 44°C indicates the presence of *Escherichia coli*. The tube at

35°C is incubated for 24 hours. If no fermentation occurs, the primary fermentation is assumed to be due to organisms other than coliforms. False positives are not uncommon in the primary fermentation tubes, due to fermentation of the sucrose in the added ice-cream by organisms other than coliforms.

After the two tubes of Lauryl Tryptose Broth have been inoculated for secondary fermentation, test the original primary fermentation tube (which was inoculated directly with ice-cream) for indole production. A positive reaction suggests the presence of *E. coli* and confirmation will be obtained later with the secondary fermentation from the 44°C bath. A negative indole reaction in the primary fermentation tube indicates the absence of *E. coli.*

MUG Reagent BR71 – The addition of 4-methylumbelliferyl-β-D-glucuronide (MUG) BR71 to this medium will enhance the detection of *Escherichia coli*.

The use of MUG in a Most Probable Number (MPN) technique for enumeration of presumptive *E. coli* in milk and milk products has been specified in a standard procedure[8]. For further information about MUG see MUG Reagent BR71 under Biological Reagents.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at room temperature (18–22°C).

Quality Control
Positive control:
Escherichia coli ATCC® 25922
(Gas 35°C and indole 44°C)
Enterobacter aerogenes ATCC® 13048
(Gas 35°C and no indole at 44°C)

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions
If stored at 2–8°C the broth will become cloudy or form a precipitate. This should clear at room temperature but gas formation is the criterion of growth not turbidity.

References

1 American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington DC.*
2 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.*
3 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.*
4 MacConkey A. T. (1938) *J. Hyg. 8. 322–334.*
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LEGIONELLA SELECTIVE MEDIA

For the isolation of Legionellaceae from clinical and environmental samples.

LEGIONELLA CYE AGAR BASE

Code: CM655

Charcoal Yeast Extract Agar for the isolation of Legionellaceae when used with Legionella BCYE Growth Supplement SR110 (Edelstein BYCE Agar).

Formula	gm/litre
Activated charcoal	2.0
Yeast extract	10.0
Agar	13.0

LEGIONELLA BCYE GROWTH SUPPLEMENTS

Supplement containing *L-cysteine.*

Code: SR110

SR110A Vial contents (each vial is sufficient for 100ml of medium)

	gm/litre
ACES Buffer/Potassium hydroxide	10
Ferric pyrophosphate	0.25
L-cysteine HCl	0.4
α-Ketogluterate	1

SR110C Vial contents (each vial is sufficient for 500ml of medium) Supplement omitting *L-cysteine*, for presumptive identification of *Legionella* spp.

Code: SR175
Vial contents (each vial is sufficient for 100ml of medium)

	gm/litre
ACES Buffer/Potassium hydroxide	10
Ferric pyrophosphate	0.25
L-cysteine hydrochloride	Nil
α-Ketoglutarate	1.0

LEGIONELLA BMPA SELECTIVE SUPPLEMENT

Code: SR111

Vial contents (each vial is sufficient for 100ml of medium)

Cefamandole	400µg
Polymyxcin B	8,000 IU
Anisomycin	8mg

LEGIONELLA MWY SELECTIVE SUPPLEMENT

Code: SR118

Vial contents (each vial is sufficient for 100ml of medium).

Glycine	0.3g
Polymyxin	5,000 IU
Anisomycin	8.mg
Vancomycin	100µg
Bromothymol blue	1mg
Bromocresol purple	1mg

Directions to prepare BCYE Agar
Suspend 2.5g of Legionella CYE Agar Base in 90ml of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Allow to cool to 50°C and aseptically add the contents of one vial of Legionella BCYE Growth Supplement SR110 reconstituted in 10ml of warm sterile distilled water (50°C). Mix gently and pour into sterile petri dishes. The final pH of the medium will be 6.9 ± 0.1.

Directions to prepare BMPAα, MWY and GVPC Legionella Selective Media
Suspend 2.5g of Legionella CYE Agar Base in 90ml of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Allow to cool to 50°C and aseptically add the contents of one vial of Legionella BYCE Growth Supplement SR110 and one vial of either Legionella BMPAα Selective Supplement SR111, reconstituted in 2ml of sterile distilled water. Mix gently and pour into sterile petri dishes. The final pH of both media will be 6.9 ± 0.1.

Description
The discovery of the causative organism of Legionnaires' disease has been reviewed by Fallon[1]. Since that review further progress has been made in culturing the organism from clinical specimens and also in the enumeration of *Legionella* species from environmental samples. Feeley et al.[2] described a modification of F-G Agar[3] in which acid hydrolysed casein was replaced by yeast extract as the source of protein and starch was replaced by activated charcoal (Norit A) at a final concentration of 0.2% (w/v). This medium, which they named CYE Agar[2] has been further supplemented with ACES Buffer and α-ketogluterate and is described in the literature as BCYE-α Medium[4]. BCYE-α Medium has been shown to yield optimal recovery of Legionellaceae in a shorter incubation period from environmental samples and clinical specimens[5].

Oxoid BCYE Medium is based on the formulation of Edelstein[4] and is prepared from Legionella CYE Agar Base CM655 and Legionella BCYE Growth Supplement SR110. The sterile lyophilised supplement contains ACES Buffer/potassium hydroxide, α-ketogluterate, ferric pyrophosphate and L-cysteine HCl. When added to CYE Agar Base it stabilises the pH of the medium at 6.9 ± 0.1 and provides essential growth factors.

Additionally, a medium omitting L-cysteine may be prepared from Legionella CYE Agar Base CM655 and BYCE Growth Supplement SR175.

Legionellaceae have an absolute nutritional requirement for L-cysteine. Presumptive *Legionella* spp. colonies can be subcultured onto both BCYE Medium with L-cysteine CM655 and SR110, and BCYE Medium without L-cysteine CM655 and SR175.

All plates are incubated at 35°C. Colonies which have grown on BCYE Medium with L-cysteine, but not on BCYE Medium without L-cysteine, can be regarded as presumptive *Legionella* spp.

Quality Control

	Legionella pneumophila ATCC® 33153	*Escherichia coli* ATCC® 25922
SR175	–	+
SR110	+	+

Edelstein and Edelstein later compared agars used in the manufacture of buffered charcoal yeast extract medium and found significant differences in growth occurred. Oxoid Agar L11 gave the best results in the series tested[6].

1 **BMPAα Medium** containing polymyxin 80 IU/ml, anisomycin 80mg/ml and cefamandole 4mg/ml. This semi-selective medium was recommended by Edelstein[4] for the isolation of *L. pneumophila* from contaminated clinical and environmental specimens and can be prepared by supplementing BCYE Agar with Legionella BMPAα Selective Supplement SR111.

2 **Wadowsky and Yee Medium[7] modified by Edelstein (MWY Medium)**[8] containing polymyxin 50 IU/ml, anisomycin 80mg/ml, vancomycin 1mg/ml and glycine 0.3% w/v. Bromocresol purple 10mg/ml and Bromothymol blue 10mg/ml colour the colonies and aid in the identification of the organisms[9,10]. Edelstein[8] considered the medium to be the best for isolating *L. pneumophila* from potable water samples. This medium can be prepared by supplementing BCYE Agar with Legionella MWY Selective Supplement SR118.

   Environmental samples should be pre-treated with an acid buffer (pH 2.2)[11] or by heat treatment[12]. They should be plated both before and after treatment to maximise recovery (see Technique).

   MWY medium has been used successfully for examination of clinical specimens[5].

3 **GVPC Medium** based on the formula by Dennis et al. This medium contains glycine, vancomycin, polymyxin B and cycloheximide. A complete description follows on page 2-123.

Technique: Clinical Samples
For the isolation of *Legionella* spp. from patients with clinical evidence of Legionnaires' disease, greatest success has been achieved by the examination of lung tissue and bronchial aspirate.

1 Homogenise the patient's specimen in sterile distilled water.

2 Examine microscopically for Legionella by the Fluorescent Antibody Method (FA) and for other bacteria by Gram's stain.

3 Inoculate specimens that are FA-positive but with no other organisms present on to plates of BCYE Medium. Both FA-positive and FA-negative specimens in which other organisms have been detected by the Gram stain, are inoculated on to the selective medium BMPAα.

4 Incubate the plates at 35°C in a 90% relative humidity atmosphere.

5 Growth usually appears in 2–3 days but continue to examine daily for 14 days before discarding the plates.

Environmental Samples

1 Take 10ml of the concentrated sample and centrifuge at 2,500 rpm for 20 minutes (using sealed buckets).

2 Remove the supernatant to leave approximately 1ml of fluid. Resuspend the deposit. This constitutes the inoculum.

3 Spread 0.1ml on to plates of BCYE Medium with and without selective agents using a sterile spreader.

4 Add 9ml of HCl-KCl buffer* (pH 2.2); shake gently and leave for 5 minutes.

*HCl-KCl buffer:
3.9ml of 0.2 M HCl
25ml of 0.2 M KCl
Adjust the pH to 2.2 using 1M KOH.

Alternatively, heat 10ml of the sample concentrate in a 50°C water bath for 30 minutes.

Important
ACID AND HEAT PRETREATMENT OF SAMPLES MUST NOT BE COMBINED.

5 Spread 0.1ml on to plates of BCYE Medium using a sterile spreader.

6 Incubate the plates at 35°C and examine daily for up to seven days.

Colonies suspected of being Legionella are subcultured to Tryptone Soya Agar containing 5% sheep blood and BCYE Agar.

Isolates that grow on BCYE Agar but fail to grow on TSA Blood Agar and have characteristic morphology, may be presumed to be *Legionella*. Confirmation must be made by biochemical and serological tests[12].

As the media described are not completely selective for *Legionella* species, it is recommended[8] that the following criteria are used for the examination of plates:

1 the colonies must have characteristic colour, size and appearance when examined under a dissecting microscope.

2 the isolates should not grow on blood agar.

3 the organisms should show characteristic Gram morphology.

Legionella spp. cannot be identified solely on growth characteristics on various media or by biochemical tests. Further studies with DNA homology, cellular fatty acids and serotyping must be undertaken.

Colony morphology after incubation at 35°C for 2–3 days on CYE/BCYE media

L. pneumophila: diameter 1–2mm (increase in size on further incubation). White, glistening, circular, smooth, raised with an entire edge.

L. gormanii: diameter 1–2mm Buff-white or cream, slight raised, mucoid.

Other legionellae: *(L. micdadei, L. bozemanii, L. dumoffii, L. longbeachae* and *L. jordanis)* – indistinguishable from *L. pneumophila.*

Characteristics of *Legionella* species (Adapted From Renner & Tseng[10])

Characteristic	*L. pneumophila*	*L. bozemanii*	*L. dumoffii*	*L. micdadei*	*L. gormanii*	*L. longbeachae*	*L. jordanis*
Primary isolation on:							
BCYEα Agar	+	+	+	+	+	+	+
Blood Agar	–	–	–	–	–	–	–
Colony colour on dye containing medium	white-green	blue-grey	green	green	green	white-green	white-green
Gram reaction	–ve	–ve	–ve	–ve	–ve	–ve	–ve
Acid fast in tissue	–	–	–	–	–	–	–
Flagella	+	+	+	+	+	+	+
Oxidase	+	–	–	+	–	+	+
Catalase	+	+	+	+	+	+	+
Beta-lactamase	+	+	+	–	+	+	+

Characteristic Gram morphology on first isolation
Gram-negative, short, pleomorphic rods 1μm long.

Carbol-fuchsin should be used as a counterstain in the Gram films of legionellae because they resist staining with safranin and basic fuchsin.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C away from light.

Quality Control
Positive control:
Legionella pneumophila ATCC® 33152
Legionella pneumophila NCTC 12821

Negative control:
Escherichia coli ATCC® 25922
Staphylococcus epidermidis ATCC® 12228

Please note for Legionella MWY Selective Supplement SR118, *Escherichia coli* ATCC® 25922 as a negative control is inoculum dependent.

Precautions
Legionella spp. are highly pathogenic organisms if inhaled. Avoid creating aerosols and handle liquid cultures or suspensions of organisms in a protective cabinet. Decontaminate working surfaces with 5% hypochlorite. Autoclave all materials before discarding.

Incubate cultures at 35°C in 65% humidity up to 10 days.

No CO_2 is required for BCYE cultures but 2.5% CO_2 is recommended for other media and water samples[7,13].

Organisms that grow on blood agar as well as Legionella media are not legionellae. Some thermophilic spore-bearing organisms mimic *Legionella* colonies after 35°C incubation. These organisms can be detected by incubating parallel plates at 35°C and 55°C when the thermophilic organisms will grow at the higher temperature. *Legionella* species will not grow above 45°C.

References

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LEGIONELLA (GVPC) SELECTIVE SUPPLEMENT

Code: SR152

A freeze-dried selective supplement for the isolation of Legionella spp. from environmental water samples.

Vial contents (each vial is sufficient to supplement 500ml of BCYE Medium).

Glycine (Ammonia free)	1.5g
Vancomycin hydrochloride	0.5mg
Polymixin B sulphate	39600 IU
Cycloheximide	40.0mg

Directions
To one vial aseptically add 10ml of sterile distilled water and mix gently to dissolve completely. Add the contents to 500ml of sterile BCYEα Medium (prepared using Legionella CYE Agar Base CM655 to which one vial of Legionella BCYEα Growth Supplement SR110C has been added), cooled to 50–55°C. Mix gently and pour into sterile petri dishes.

Description
L. pneumophila was first isolated by infecting guinea-pigs and fertile hens' eggs in 1977[1]. Examination of water samples for the presence of legionellae has frequently been undertaken using direct immunofluorescence methods[2]. However, isolation is still the most widely accepted method of demonstrating the presence of Legionellae and is of particular value in epidemiological studies.

Currently, a buffered charcoal yeast extract medium (BCYE) is generally considered to be the medium of choice[3], to which various antimicrobials have been added to make it selective.

GVPC Selective Supplement is based on the formulation described by Dennis et al[4]. This selective formulation has been reported to be the most efficient *in vitro* method for the isolation of *L. pneumophila* when used in conjunction with acid or heat pretreatments. Cycloheximide is included because it has a greater activity against fungi than anisomycin which is almost exclusively active against yeasts. Fungi commonly occur more frequently than yeasts in environmental waters examined for Legionella. This formulation is to be specified by the BSI (British Standard) for the detection and enumeration of legionellae in water and related materials[5].

The antimicrobials Glycine, Vancomycin and Polymixin will collectively inhibit most Gram-positive and Gram-negative bacterial growth. Cycloheximide will suppress the growth of fungi.

Technique
For each sample, three plates should be inoculated[3,5]: one after pretreatment with heat, one after pretreatment with acid and one that has received neither pretreatment.

Heat pretreatment
1 Take 10ml of concentrated sample[3] and place in a water bath at 50°C for 30 minutes.

Acid pretreatment
1 Take 10ml of concentrated sample[3] and centrifuge in sealed buckets at 2,500rpm for 20 minutes.

2 Decant the supernatant to leave approximately 1ml of fluid.

3 Add 9ml of HCl-KCl buffer (see below) and resuspend by gentle shaking. Leave to stand for 5 minutes and inoculate without further delay.

HCl-KCl Buffer
3.9ml of 1.2M HCl
25ml of 0.2M KCl
Adjust to pH 2.2 using 1M KOH

No pretreatment

1 Take 10ml of concentrated sample[3] and do not pretreat.

Directions

1 Spread 0.1ml of each portion, described above, onto plates of GVPC Selective Medium using a sterile spreader.

2 Incubate the plates at 36 $\pm$ 1°C and examine on days 3, 5, 7 and 10.

3 Suspect colonies should be verified as presumptive Legionella by the following procedure: Select several colonies of each type and subculture on to a pair of plates, one of Buffered Charcoal Yeast Extract Agar CM655 and one of Buffered Charcoal Yeast Extract Agar CM655 containing Legionella BCYE Growth Supplement SR110. Regard as presumptive Legionella all colonies which grow on Buffered Charcoal Yeast Extract Agar containing the supplement but which fail to grow on medium not containing the supplement.

4 Further confirmation of identity can be obtained by subculturing colonies taken from the primary culture plates on to Blood Agar. Isolates that fail to grow on Blood Agar and are small, poorly staining Gram-negative rods are presumptively identified as *Legionella* species.

5 Each presumptive *Legionella* colony should be confirmed by serology.

L. pneumophila colonies are white-grey-blue in appearance and up to 2mm in diameter with a ground-glass appearance. Colonies of all *Legionella* spp. exhibit the same general appearance but they may differ in colour. Colours include brown, lime green, deep red and blue-purple.

Storage conditions and Shelf life

Store the selective supplement in the dark at 2°C to 8°C and use before the expiry date on the label.

Quality Control

The medium may be tested for performance using stable typical control cultures of organisms other than Legionellae. However, when testing Legionellae, 'wild' strains must be used because Legionellae easily become adapted to growth under laboratory conditions and will grow on media that would not support the primary isolation of 'wild' strains[5]. 'Wild' strains can be defined as strains which have been subcultured no more than twice following primary isolation. GVPC Medium should not be tested using *L. pneumophila* strains that may have become laboratory acclimatised.

Incubation at 35–37°C $\pm$ 0.5°C for 5 days in air or in air enriched with 2.5% CO_2[5] in conditions of high humidity.

Positive control:
Legionella species

Negative control:
Staphylococcus epidermidis ATCC® 12228
Escherichia coli ATCC® 25922

References

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LISTERIA SPECIES AND LISTERIOSIS

Listeriosis is a disease which has been recognised for over 60 years, it is now known to affect a wide variety of animals as well as humans.

There are eight species in the *Listeria* genus:

L. monocytogenes
the major species causing disease in humans and animals[1].

L. ivanovii
associated with animal and a small number of human infections[2].

L. innocua, L. seeligeri
very rarely associated with infection.

L. murrayii, L. welshimeri, L. denitrificans
not associated with naturally occurring infections.

L. monocytogenes is commonly found in the environment and is therefore common in food and human faeces. It can grow slowly at refrigerator temperatures and has been isolated from a wide variety of foods. Fresh and frozen poultry (60%), cooked-chill foods (24%), salami and continental sausages (24%), soft cheese (10%), pre-packed salads (7%)[3]. Although relatively few cases of listeriosis have been epidemiologically associated with food stuffs in the UK[3,4] a number of outbreaks associated with food have been reported in other countries[5,6,7,8]. Caution should be taken in assessing food-borne infections because the extent and route of such infections are unknown.

The annual number of cases of listeriosis reported to the Central Public Health Laboratory has risen from 50 in 1967 to 259 in 1987. Reported deaths are 38 in 1983 rising to 59 in 1987[3]. In human listeriosis there is a high rate of mortality (23%) and the disease is largely confined to pregnant women, neonates and patients with underlying immuno-suppression[2].

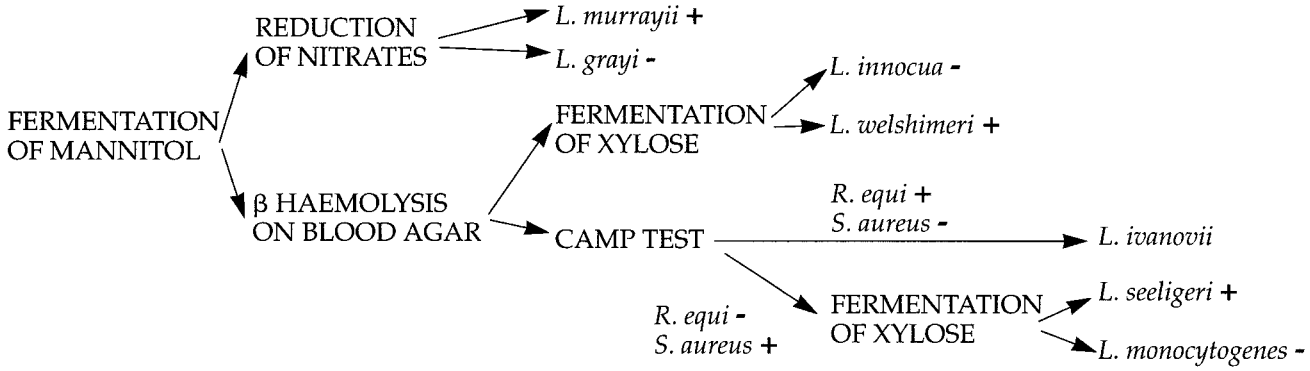
The use of efficient, selective media will undoubtedly improve the rate of isolation and may reveal the chain of infection which is still very obscure.

TABLE 1 **Differentiation of the species of the genus *Listeria***

	L. monocytogenes	*L. ivanovii*	*L. innocua*	*L.welshimeri*	*L. seeligeri*	*L. grayi*	*L. murrayi*	*L. denitrificans*
Beta haemolysis on blood agar	+	++	–	–	(+)	–	–	–
Nitrates reduced to nitrites	–	–	–	–	–	–	+	+
CAMP test with *Staphylococcus aureus*	+	–	–	–	(+)	–	–	–
CAMP test with *Rhodococcus equi*	–	+	–	–	–	–	–	–
Acid produced from:								
D-mannitol	–	–	–	–	–	+	+	–
L-rhamnose	+	–	V	V	–	–	V	–
D-xylose	–	+	–	+	+	–	–	+
α-methyl D-mannoside	+	–	+	+	V	NS	NS	NS

V = Variable reaction; NS = Not stated; ( ) = Weak reaction

FIGURE 1 **Dichotomous key for the identification of the species of *Listeria***



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IDENTIFICATION OF LISTERIA SPECIES

(adapted with permission from reference 1.)

Criteria of identification for Listeriae
Gram positive rod, VP +, urease –, catalase +, oxidase – aesculin hydrolised, acid no gas from glucose and salicin, tumbling motility below 30°C.

Colony form – non pigmented on nutrient agar with characteristic caramel/sour butter smell, 1–2mm diameter with a ground glass appearance and emulsifies in saline to a smooth suspension.

Motility test – heavily inoculate brain-heart infusion broth or nutrient broth and hold at *room temperature* for 4–6 hours. Examine by hanging drop technique. If negative re-test after 18 hours at room temperature.

Catalase test – most strains are strongly positive but some may be weak or negative.

Haemolysis – *L. monocytogenes* produces a narrow zone of β-haemolysis (1–2mm) on horse blood agar.

CAMP test – use 5% v/v sheep blood in nutrient agar and pour a thin layer over nutrient agar base. Streak *Staphylococcus aureus* NCTC 1803 and *Rhodococcus equi* NCTC 1621 across the plates. The test strains of listeriae are streaked at right angles to the *S. aureus* or *R. equi* inoculum. Incubate at 35°C overnight. Positive results are when an enhanced zone of haemolysis occurs between two cultures.

VP test – some strains may require yeast extract added to the VP broth to give sufficient growth for this test.

Carbohydrate fermentation – use API 50 CH system. Incubate up to 7 days at 35°C.

Nitrate reduction – Use the rapid method of Blazeric D. N. & Ederer G. M. 'Biochemical Tests in Diagnostic Bacteriology' J. Wiley & Son Inc. New York. 1975.

Acknowledgement is gratefully made to the Central Public Health Laboratory for permission to use this information and for Table 1 with Figure 1.

BUFFERED LISTERIA ENRICHMENT BROTH

Code: CM897

A selective enrichment medium for the detection of Listeria monocytogenes when prepared from Buffered Listeria Enrichment Broth base CM897 and Listeria Selective Supplement SR141.

BUFFERED LISTERIA ENRICHMENT BROTH CM897

Formula	gm/litre
Tryptone soya broth	30.0
Yeast extract	6.0
Potassium di-hydrogen orthophosphate	1.35
Disodium hydrogen orthophosphate	9.60
Final pH 7.3 ± 0.2	

LISTERIA SELECTIVE ENRICHMENT SUPPLEMENT (SR141)

Vial contents:

Nalidixic acid	20.0mg	(equivalent to 40mg/l)
Cycloheximide	25.0mg	(equivalent to 50mg/l)
Acriflavine hydrochloride	7.5mg	(equivalent to 15mg/l)

Each vial is sufficient to supplement 500mls of Buffered Listeria Enrichment Broth CM897.

Directions
Add 23.5g to 500ml of distilled water and mix well to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of 1 vial of Listeria Selective Enrichment Supplement SR141 reconstituted with 2ml of sterile distilled water. Mix well and aseptically distribute into sterile containers in volumes as required.

Description
Listeria Selective Enrichment Broth CM862 is based on the formulation described by Lovett et al[1] and is recommended for the enrichment of *Listeria* species in food. Subsequent work has concluded that the enrichment properties can be improved by increasing the buffering capacity of the medium by the addition of potassium di-hydrogen orthophosphate and disodium hydrogen orthophosphate. Buffered Listeria Enrichment Broth CM897 is therefore a modification of the original medium.

Techniques
1 Add 25g or 25ml samples to 225ml of Buffered Listeria Enrichment Broth. Homogenise if required.
2 Incubate at 30°C for 48 hours.
3 Subculture from the Buffered Listeria Emrichment Broth onto Listeria Selective Agar plates (See Note) after 24 and 48 hours by:
   (i) Direct plating onto Listeria Selective Agar plates.
   (ii) Adding 1ml of the Buffered Listeria Enrichment Broth to 9ml of 0.5% KOH, vortex mixing, and plating onto Listeria Selective Agar plates.

Note
Suitable Listeria Selective Media are:
1 Listeria Selective Medium (Oxford formulation) (Oxoid CM856 and Oxoid SR140).
2 Listeria Selective Medium (MOX) (Oxoid CM856 and Oxoid SR157)
3 PALCAM Medium (Oxoid CM877 and Oxoid SR150)

Quality Control
Positive Control:
Listeria monocytogenes ATCC®19117

Negative Control:
Staphylococcus aureus ATCC®25923

Storage conditions and Shelf life
Buffered Listeria Enrichment Broth CM897 should be stored tightly capped in the original container in a cool, dry place away from bright light. When stored as directed the medium will remain stable until the expiry date printed on the label.

Listeria Selective Enrichment Supplement SR141 as supplied should be stored at 2–8°C. When stored as directed the reagents are stable until the expiry date printed on the label.

Precautions
Listeria Selective Enrichment Supplement contains cycloheximide and is toxic if swallowed, inhaled or by skin contact. As a precaution when handling wear gloves and eye/face protection.

Acriflavine is activated by light and may form compounds inhibitory for Listeria.

References

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LISTERIA SELECTIVE AGAR

(OXFORD FORMULATION)

Code: CM856

A selective and diagnostic medium for the detection of Listeria monocytogenes, when prepared from Listeria Selective Agar Base CM856 and Listeria Selective Supplement SR140.

Formula	gm/litre
Columbia Blood Agar Base	39.0
Aesculin	1.0
Ferric ammonium citrate	0.5
Lithium chloride	15.0
pH 7.0 $\pm$ 0.2	

LISTERIA SELECTIVE SUPPLEMENT (OXFORD FORMULATION)

Code: SR140

Vial contents (each vial is sufficient for 500ml of medium)

Cycloheximide	200mg
Colistin sulphate	10mg
Acriflavine	2.5mg
Cefotetan	1.0mg
Fosfomycin	5.0mg

Directions

Suspend 27.75 of the Listeria Selective Agar Base (Oxford Formulation) CM856 in 500ml of distilled water. Bring gently to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of Listeria Selective Supplement (Oxford Formulation) SR140 reconstituted with 5ml of ethanol/sterile distilled water (1:1). Mix well and pour into sterile petri dishes.

Prepared plates may be stored for up to 10 days at 4°C in the dark room.

Description

Foodborne infection by *Listeria monocytogenes* has prompted increased concern for detecting this organism in foods, in the environment and in pathological specimens from both human and animal subjects.

Most infections in adult humans are symptomless and result in intestinal, vaginal and cervical carriage. Infection during pregnancy may cause abortion, premature delivery and neonatal infection. The possibility of listeriosis should be considered in any woman with unexplained recurrent miscarriage, premature labour or foetal death. The organism should be sought in blood cultures and genital-tract swabs[1].

The most common clinical manifestation in both adults and neonates is meningitis. Widely disseminated infection, abscesses, sub-acute bacterial endocarditis and opportunistic infections in immunosuppressed patients occur less frequently.

Birds, fish and other animals are all susceptible to infection with Listeria. It is of particular importance in domestic farm animals. In the Federal Republic of Germany reporting of listeriosis in animals is compulsory and meat inspection law in the same country requires examination for Listeria because of its significance in meat hygiene.

Listeria monocytogenes is very widespread in the environment. Isolation has been reported from milk[2,3], cheese[4], sewage and riverwater[5], and silage[6]. Because Listeria is so widespread sources of infections are numerous. Uncooked vegetable foods have been implicated; an episode associated with consumption of coleslaw[7] was linked with cabbage from a farm using sewage fertiliser. In outbreaks caused by dairy products, cattle with mastitis may be the source of the organism. Of great importance to veterinarians is the considerable increase amongst sheep of infection manifesting as abortion or encephalitis due largely to changing practices in silage manufacture[8].

The ability to isolate the organism has been impeded in the past by lack of an effective selective medium, as *L. monocytogenes* can be easily and completely overgrown by competing flora.

Listeria Selective Medium (Oxford Formulation) is based on the formulation described by Curtis et al[9] and is recommended for the detection of *Listeria monocytogenes* from clinical and food specimens.

The medium utilises:

(i) the selective inhibitory components lithium chloride, acriflavine, colistin sulphate, cefotetan, cycloheximide and fosfomycin, and

(ii) the indicator system aesculin and ferrous iron for the isolation or differentiation of *L. monocytogenes.*

L. monocytogenes hydrolyses aesculin, producing black zones around the colonies due to the formation of black iron phenolic compounds derived from the aglucon. Gram-negative bacteria are completely inhibited. Most unwanted gram-positive species are suppressed, but some strains of enterococci grow poorly and exhibit a weak aesculin reaction, usually after 40 hours incubation. Some staphylococci may grow as aesculin-negative colonies.

Typical *L. monocytogenes* colonies are almost always visible after 24 hours, but incubation should be continued for a further 24 hours to detect slow-growing strains.

Techniques for isolation vary with the author and the material under examination[10,11]. For all specimens selective enrichment and cold enrichment have been shown to increase isolation rates significantly[12,13,14]. The efficacy of Listeria Selective Medium (Oxford Formulation) has been confirmed for various foods[15,16] following the methodology and using

selective enrichment media described in the literature[16,17,18,19].

Oxford agar is a specified plating medium in the FDA/BAM isolation procedure[20] and in the standardised testing methods of other national and international bodies[21].

Oxford agar base was used by Al-Zoreki and Sandine as the basal medium for their ASLM agar which incorporates ceftazidime, moxalactam and cycloheximide as selective agents[22].

Technique

Faecal and Biological Specimens

The sample is homogenised in 0.1% Peptone Water CM9 (1 part to 9 parts peptone water).

Direct Surface Plate Method

1 Inoculate 0.1ml of the homogenised specimen onto the Listeria Selective Medium plates.
2 Incubate at 35°C for up to 48 hours.
3 Examine for typical colonies of Listeria after 24 and 48 hours incubation.

Selective Enrichment Method

1 Add the homogenised specimen to the selective enrichment broth and incubate at 30°C for up to 7 days.
2 Inoculate 0.1ml of the selective enrichment broth, after 24 hours, 48 hours and 7 days, onto the Listeria Selective Medium plates.
3 Incubate the plates at 35°C for up to 48 hours.
4 Examine for typical colonies of Listeria after 24 and 48 hours incubation.

Food and Environmental Samples

Techniques for isolation vary with the author, material and authorities. For detection of *L. monocytogenes* when present in small numbers, the test samples must be inoculated into an enrichment broth to allow multiplication before isolation and identification. Depending on the type of sample under test, an appropriate method and selective enrichment broth should be chosen prior to inoculation onto the Listeria Selective Medium plates.

1 Inoculate 0.1ml of the selective enrichment broth onto the Listeria Selective Medium plates.
2 Incubate at 35°C for up to 48 hours.
3 Examine for typical colonies after 24 and 48 hours incubation.

Colonies presumptively identified as *L. monocytogenes* must be confirmed by biochemical and serological testing[23].

Note

Differences in susceptibility of *L. monocytogenes*, *L. seeligeri* and *L. ivanovii* to β-lactam antibiotics and fosfomycin have been observed dependent on whether incubation is at 30°C or 35–37°C[24].

Storage conditions and Shelf life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control

Positive control:
Listeria monocytogenes ATCC® 19117

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions

L. monocytogenes is in ACDP Group 2 i.e. 'might be a hazard to laboratory workers' and should be handled in a suitable environment only. It is also recommended that pregnant staff should be excluded from working with known cultures of listeriae.

Listeria media containing acriflavine should be protected from light because photo-oxidation makes it inhibitory to listeria.

Supplement SR140 used in this medium contains a toxic concentration of cycloheximide. Note the precautions to be taken under HAZARDS page 2-7.

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LISTERIA ENRICHMENT BROTH BASE

Code: CM862

Formula	gm/litre
Tryptone soya broth	30.0
Yeast extract	6.0
pH 7.3 ± 0.2	

LISTERIA SELECTIVE ENRICHMENT SUPPLEMENT

Code: SR141

Vial contents (each vial is sufficient for 500ml of medium)

Nalidixic acid	20.0mg
Cycloheximide	25.0mg
Acriflavine hydrochloride	7.5mg

Directions
Suspend 18g in 500ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of Listeria Selective Enrichment Supplement SR141, reconstituted with 2ml of sterile distilled water. Mix well and distribute into sterile containers in volumes as required.

Listeria Selective Enrichment Supplement (modified with 10 mg/litre of Acriflavine) Code: SR149.

Vial contents (each vial is sufficient to supplement 2.25 litres of CM862).

Nalidixic acid	90.0mg
Cycloheximide	112.5mg
Acriflavine hydrochloride	22.5mg

Directions
Aseptically add 10ml of sterile distilled water to one vial and invert gently to dissolve. Aseptically add the vial contents to 2.25 litres of Listeria Enrichment Broth Base (CM862), cooled to 50°C.

Description
Listeria Selective Enrichment Medium is based on the formulation described by Lovett et al.[1] and is recommended for the selective enrichment of *Listeria* species from food. The enrichment procedure has been shown to recover an inoculum of less than 10cfu/ml from raw milk.

In order to achieve a higher isolation rate it is recommended that the enrichment broth is subcultured onto Listeria Selective Agar plates after 1, 2 and 7 days. Agello et al.[2], have shown that extending the incubation period to 7 days allows better recovery of environmentally stressed listeria from milk and milk products.

Technique
1 Add 25g or 25ml samples to 225ml of Listeria Selective Enrichment Broth. Homogenise if required.
2 Incubate at 30°C for 7 days.
3 Subculture from the Listeria Selective Enrichment Broth onto Listeria Selective Agar plates (see Note) after 1, 2 and 7 days by:
   (i) Direct plating onto Listeria Selective Agar plates.
   (ii) Adding 1ml of the Listeria Selective Enrichment Broth to 9ml of 0.5% KOH, vortex mixing, and plating onto Listeria Selective Agar plates.

Note:
Suitable Listeria Selective Media are:

1 Listeria Selective Medium (Oxford formulation) (Oxoid CM856 and Oxoid SR140).
2 Listeria Selective Medium (MOX) (Oxoid CM856 and Oxoid SR140).
3 PALCAM Medium (Oxoid CM877 and Oxoid SR150).

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Listeria monocytogenes ATCC® 19117

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions
Note the precautions stated under Listeria Selective Medium (Oxford) CM856 and SR140. Broth cultures are more dangerous than colonies on agar plates.

Store prepared medium away from light. Acriflavine can photo-oxidise to form inhibitory compounds against listeria.

Supplement SR141 used in this medium contains a toxic concentration of cycloheximide. Note the precautions to be taken under HAZARDS page 2–7.

References
1 Lovett J., Francis D. W. and Hunt J. M. (1987) *Journal of Food Protection 50. 188–192.*
2 Agello G., Hayes P. and Feeley J. (1986) *Abstracts of the Annual Meeting, ASM, Washington DC p5.*

LISTERIA ENRICHMENT BROTH BASE

(UVM FORMULATION)

Code: CM863

Formula	gm/litre
Proteose peptone	5.0
Tryptone	5.0
'Lab-Lemco' powder	5.0
Yeast extract	5.0
Sodium chloride	20.0
Disodium hydrogen phosphate	12.0
Potassium dihydrogen phosphate	1.35
Aesculin	1.0
pH 7.4 ± 0.2	

LISTERIA PRIMARY SELECTIVE ENRICHMENT SUPPLEMENT (UVM I)

Code: SR142

Vial contents (each vial is sufficient for 500ml of medium)

Nalidixic acid	10.0mg
Acriflavine hydrochloride	12.5mg

Directions
Suspend 27.2g in 500ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C.

To Prepare Listeria Primary Selective Enrichment Medium (UVM I)
Aseptically add 2ml of sterile distilled water to a vial of Listeria Primary Selective Enrichment Supplement (UVM I) Code SR142. Invert gently to dissolve. Aseptically add the vial contents to 500ml of sterile Listeria Enrichment Broth Base (UVM formulation) Code CM863, cooled to 50°C. Mix well and distribute into sterile containers.

To Prepare Listeria Secondary Selective Enrichment Medium (UVM II)
Aseptically add 2ml of sterile distilled water to a vial of Listeria Secondary Selective Enrichment Supplement (UVM II) Code SR143. Invert gently to dissolve. Aseptically add the vial contents to 500ml of sterile Listeria Enrichment Broth Base (UVM formulation) Code CM863, cooled to 50°C. Mix well and distribute into sterile containers.

Description
The Listeria Selective Enrichment Media (UVM formulations) are based on the original formulation described by Donnelly and Baigent[1], and its subsequent modification[2] which reduced the nalidixic acid concentration in both the primary and secondary selective enrichment media and increased the concentration of acriflavine hydrochloride in the secondary selective enrichment medium.

This modification, and the two step selective enrichment method developed (USDA-FSIS method)[2], results in a higher detection rate of *Listeria monocytogenes* from meat products and has the added advantage of only taking 3–4 days.

UVM Broth has been recommended as a primary enrichment broth for recovery of heat-injured *L. monocytogenes*[3].

Care must be taken when using UVM broth with DNA probe methodology because the high salt content of the medium may have an inhibitory effect on detection[4].

Technique
Primary Enrichment
1 Add 25g or 25ml samples to 225ml of Listeria Primary Selective Enrichment Medium (UVM I). Homogenise in a Stomacher for 2 minutes.
2 Incubate the prepared sample in the Stomacher bag at 30°C.
3 From this bag, carry out the following procedures:
After 4 hours incubation, spread 0.2ml on Listeria Selective Agar plates (see Note).
After 24 hours incubation,
(i) transfer 0.1ml to 10ml of Listeria Secondary Enrichment Medium (UVM II), and
(ii) transfer 1ml to 4.5ml KOH solution. Vortex mix and within one minute subculture onto Listeria Selective Agar plates. For details of KOH preparation see below.

Secondary Enrichment
4 Incubate the inoculated Listeria Secondary Selective Enrichment Medium (UVM II) at 30°C. See 3(i).
5 After 24 hours incubation,
(i) spread 0.2ml onto Listeria Selective Agar plates.
(ii) transfer 1ml to 4.5ml KOH solution. Vortex mix and within one minute subculture this mixture onto Listeria Selective Agar plates.

Preparation Of KOH Solution
Dissolve 2.5g of KOH and 20g of NaCl in 1000ml of distilled water. Sterilise by autoclaving at 121°C and ensure that the pH is above 12.0 before use.

Note
The Listeria Selective Agar recommended for use in the USDA method[2] is LPM plating medium[3]. However, Oxoid laboratory studies[5] have shown that comparable results can be achieved with Listeria Selective Medium (Oxford formulation) CM856 and SR140.

Updated USDA methodology[6] has replaced LPM medium with Modified Oxford Medium (MOX). There is no longer a requirement to treat enrichment culture with potassium hydroxide before plating.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Listeria monocytogenes ATCC® 19117

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions
Note the precautions stated under Listeria Selective Medium (Oxford) CM856 and SR140.

Broth cultures are more dangerous than colonies on agar plates.

Store prepared medium away from light. Acriflavine can photo-oxidise to form inhibitory compounds against listeriae.

References

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FRASER BROTH

Code: CM895

A secondary selective diagnostic enrichment medium for the isolation of Listeria spp. from food and environmental specimens.

Formula	gm/litre
Proteose peptone	5.0
Tryptone	5.0
'Lab-Lemco' powder	5.0
Yeast extract	5.0
Sodium chloride	20.0
Di-sodium hydrogen phosphate	12.0
Potassium dihydrogen phosphate	1.35
Aesculin	1.0
Lithium chloride	3.0
pH 7.2 $\pm$ 0.2	

FRASER SUPPLEMENT

Code: SR156

Vial contents (each vial is sufficient to supplement 500ml of medium)

Ferric ammonium citrate	0.25g
Nalidixic acid	10.0mg
Acriflavine hydrochloride	12.5mg

Directions
Suspend 28.7g in 500ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of Fraser Selective Supplement SR156 reconstituted with 5ml of ethanol/sterile water (1:1). Mix well and distribute into sterile containers.

Description
Fraser Medium is a modification of the USDA-FSIS (United States Department of Agriculture-Food Safety Inspection Service) UVM secondary enrichment broth and is based on the formula described by Fraser and Sperber[1]. It contains ferric ammonium citrate and lithium chloride. Blackening of the medium is presumptive evidence of the presence of Listeria. Contrary to early indications, cultures which do not blacken cannot be assumed to be Listeria-free. All Fraser Broth enrichment cultures should be subcultured to plating medium.

The medium is intended for the isolation of *Listeria* spp. from food and environmental samples when used as the secondary enrichment medium in the USDA-FSIS methodology for Listeria isolation.

It is generally accepted that the USDA-FSIS two stage enrichment method employing UVM primary and secondary enrichment broths is the most suitable for the examination of meat products. Fraser Broth has proven to be remarkably accurate in detecting *Listeria* spp. in food and environmental samples[1,2].

All *Listeria* spp. hydrolyse aesculin to aesculetin. Aesculetin reacts with ferric ions which results in blackening. Another possible advantage to the addition of ferric ammonium citrate is that it has been shown that ferric ions enhance the growth of *L. monocytogenes*[3].

Lithium chloride is included in the medium to inhibit the growth of enterococci which can also hydrolyse aesculin.

Care must be taken when using Fraser Broth with DNA probe methodology because the high salt content of the medium may have an inhibitory effect on detection[4].

Technique
1 Inoculate 10ml of Fraser Broth with 0.1ml of the primary enrichment broth (i.e. FDA or UVM I enrichment broth) which has been incubated for 20 to 24 hours.
2 Incubate at 35°C for 26 $\pm$ 2 hours in air.
3 Compare each inoculated tube to an inoculated control against a white background. Tubes that darken or turn black should be subcultured on to Oxford Medium, Modified Oxford Medium (MOX) or PALCAM Medium. Tubes that retain the original yellow colour should also be inoculated on plating media and confirmed as free from *Listeria* spp. before discarding.

It should be emphasised that the incubation period should be controlled. Fraser Medium should be incubated for 26 $\pm$ 2 hours to ensure at least 24 hours incubation period to permit the development of the black colour.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the selective supplement in the dark at 2°C to 8°C and use before the expiry date on the label.

The prepared medium may be stored for up to 2 weeks at 2°C to 8°C.

Quality Control
Positive control:
Listeria monocytogenes ATCC® 19117

Negative control:
Enterococcus faecalis ATCC® 29212

References
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PALCAM AGAR BASE

Code: CM877

A selective and differential diagnostic medium for the detection of Listeria monocytogenes.

Formula	gm/litre
Columbia blood agar base	39.0
Yeast extract	3.0
Glucose	0.5
Aesculin	0.8
Ferric ammonium citrate	0.5
Mannitol	10.0
Phenol red	0.08
Lithium chloride	15.0
pH 7.2 $\pm$ 0.2	

PALCAM SELECTIVE SUPPLEMENT

Code: SR150

Vial contents

	Code: SR150E To supplement 500ml	Code: SR150B To supplement 2.5 litres
Polymixin B	5mg	25mg
Acriflavine hydrochloride	2.5mg	12.5mg
Ceftazidime	10mg	50mg

Directions
Suspend 34.5g in 500ml of distilled water. Bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of PALCAM Selective Supplement SR150, reconstituted with 2ml of sterile distilled water. Mix well and pour into sterile petri dishes.

To prepare 2.5 litres of medium, suspend 172.5g in 2.5 litres of distilled water. Sterilise and cool as above and add the contents of one vial of SR150B, reconstituted with 10ml of sterile distilled water.

The addition of 2.5% (v/v) Egg Yolk Emulsion (Oxoid code SR47) to the medium may aid the recovery of damaged Listeria.

Description
PALCAM Medium is based on the formulation described by Van Netten et al[1] and is recommended for the isolation of *Listeria monocytogenes* from foods.

The heightened awareness and concern surrounding the presence of *Listeria monocytogenes* in food has resulted in the development of many media for its isolation[2–9]. However, Cassiday and Brackett[10] conclude that no single method currently available is suitable for use with all types of food.

PALCAM Medium is highly selective due to the presence of Lithium chloride, Ceftazidime, Polymixin B and Acriflavine hydrochloride. It allows the easier differential diagnosis of *Listeria monocytogenes* by utilising the double indicator system:

1 Aesculin and ferrous iron
2 Mannitol and phenol red

Listeria monocytogenes hydrolyses aesculin resulting in the formation of a black halo around colonies. *Listeria monocytogenes* does not ferment mannitol so easy differentiation from contaminants such as enterococci and staphylococci can be made as these will ferment mannitol and produce a change from red to yellow in the pH indicator phenol red.

Incubation under micro-aerophilic conditions serves to inhibit strict aerobes such as *Bacillus* spp. and *Pseudomonas* spp. that might otherwise appear on the medium.

A modification to PALCAM medium in which incubated plates are overlaid with medium containing blood enables haemolytic *Listeria* species to be differentiated and enumerated[7].

The addition of egg yolk to PALCAM medium has been reported to aid repair of damaged cells[3].

Incubation under microaerophilic conditions serves to inhibit strict aerobes such as *Bacillus* species and *Pseudomonas* spp. that might otherwise appear on the medium.

Technique
Techniques for the isolation of *Listeria monocytogenes* will depend on the material under test. It is usual for the test sample to be first inoculated into an enrichment broth to allow multiplication before isolation and identification. Depending on the type of sample used, the appropriate method and selective enrichment broth should be used prior to inoculation onto PALCAM Medium plates. As a general rule use Listeria Selective Enrichment Medium (Oxoid codes CM862 and SR149) for dairy products and Listeria Selective Enrichment Media UVM and Fraser Broth (Oxoid codes CM863, SR142 and SR143; CM895 and SR156) for meats and poultry.

1 Inoculate one loopful of the selective enrichment broth onto the PALCAM Medium plates.
2 Incubate at 37°C for 48 hours under micro-aerophilic conditions. The micro-aerophilic condition can be best achieved by using Oxoid Campylobacter Gas Generating Kit (BR56) in conjunction with the Oxoid Anaerobic Jar and an active catalyst (BR42). For jars of smaller capacity (2.5 litres) use the Oxoid Campylobacter Gas Generating Kit (BR60). Alternatively use CampyGen CN025A or CN035A. CampyGen does not require the addition of water or a catalyst.
3 Examine for typical colonies of Listeria after 48 hours incubation.
4 Colonies identified as presumptive *Listeria* species must be confirmed by biochemical and serological testing[8].

After 48 hours incubation, typical *Listeria* spp. form colonies that are approximately 2mm in diameter, grey-green in colour with a black sunken centre and a black halo against a cherry-red medium background.

Occasional Enterococcus or Staphylococcus strains develop on PALCAM Medium to form grey colonies

with a brown-green halo or yellow colonies with a yellow halo.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the selective supplement in the dark at 2°C to 8°C and use before the expiry date on the label.

The prepared medium may be stored for up to 4 weeks at 2–8°C in the dark.

Quality Control
Positive control:
Listeria monocytogenes ATCC® 19112

Negative control:
Escherichia coli ATCC® 25922
Staphylococcus aureus ATCC® 25923
Streptococcus faecalis ATCC® 29212

Precautions
Acriflavine hydrochloride is activated by light which may cause it to become inhibitory to Listeria growth.

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LIVER BROTH

Code: CM77

A liquid medium, containing liver particles, for the examination of foods for saccharolytic or putrefactive mesophilic and thermophilic anaerobes.

Formula	gm/litre
Infusion from fresh liver	23.0
Peptone	10.0
Potassium phosphate	1.0
Extracted liver tissue	30.0
pH 6.8 ± 0.2	

Directions
Suspend 64 grams in 1 litre of distilled water and soak for 15 minutes, with occasional stirring. Distribute into 18mm diameter tubes to a depth of 50mm so that the bottom of the tube is filled with liver particles. Agitate frequently during distribution to keep the liver tissue in suspension. Sterilise by autoclaving for 20 minutes at 115°C. Inoculate when cool and then aseptically seal with a layer of sterile 2% Oxoid Agar No.3 solution.

Description
A liver infusion medium, containing liver particles, for the examination of foods for saccharolytic or putrefactive mesophilic and thermophilic anaerobes. Also recommended for the maintenance of aerobes and anaerobes in pure culture.

Technique
Gillespie in communication with Scarr[1] recommended Oxoid Liver Broth for the examination of canners' sugar for hydrogen swells caused by thermophilic anaerobes *(Clostridium thermosaccharolyticum)*. A 20% w/v solution of the sugar is steamed for 30 minutes to destroy vegetative forms and inoculated into Oxoid Liver Broth sealed with agar. The standard proposed was a maximum of 1 positive tube in six – with 20ml inocula incubated for 72 hours at 56°C.

This medium should be made up only when required for use. Storage of the reconstituted medium is not recommended because air may be absorbed and the re-steaming necessary for the restoration of anaerobic conditions darkens the medium. Liver broth is not an homogeneous medium; consisting of a layer of liver particles and a cloudy supernatant. Growth produces an obvious increase in turbidity and some organisms (e.g. *Cl. thermosaccharolyticum)* also produce gas which often pushes the agar plug towards the top of the tube. Some organisms also digest the solid liver tissue.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Clostridium thermosaccharolyticum ATCC® 7956

Negative control:
Uninoculated medium

Precautions
Note the comments on storage and reheating.

Reference

1 Scarr M. Pamela (1958) *DSIR, Proc. 2nd Internat. Symp. Food Microbiol. 1957, HMSO London, pp. 29–33.*

LYSINE DECARBOXYLASE BROTH
(TAYLOR MODIFICATION)

Code: CM308 (Tablets)

To detect lysine decarboxylase production by salmonellae and some other Enterobacteriaceae.

Formula	gm/litre
Yeast extract	3.0
Glucose	1.0
L-lysine	5.0
Bromocresol purple	0.016
pH 6.1 ± 0.2	

Directions
Add 1 tablet to 5ml of distilled water in a 1/4 oz screw-capped bottle. Sterilise by autoclaving at 121°C for 15 minutes.

Note Uninoculated the medium should be blue/grey in colour.

Description
Lysine Decarboxylase Broth is a diagnostic medium which distinguishes salmonellae (and some other Enterobacteriaceae) by a distinct biochemical reaction.

Taylor's modification of the medium[1] shows an improved performance over the formulation described by Falkow[2]. This is achieved by omitting peptone from the medium, thus eliminating false positives caused by *Citrobacter freundii* and its paracolon types. These organisms utilise peptone as a nitrogen source, produce an alkaline reaction and mask the absence of lysine decarboxylase.

Taylor's modification shares the advantages of Falkow's formulation over that of Moller[3] in that it does not require the special conditions of anaerobic culture and low pH and it is relatively easy to control.

During the initial stages of incubation, fermentation of glucose by the organism, with production of acid, results in a colour change in the indicator to yellow. On further incubation, if lysine is decarboxylated to cadaverine, there will be an alkaline reaction. The indicator colour will then change to purple (positive). If the colour remains yellow, the reaction is negative.

Decarboxylase reactions of various members of the Enterobacteriaceae on lysine

Organism	*Lysine Decarboxylation*
Salmonella species	+
S. paratyphi A	–
Shigella species	–
Escherichia coli (including late-lactose variants Alcalescens-Dispar)	V
Citrobacter species (including the Bethesda-Ballerup group)	–
Providencia species	–
Proteus species	–
Serratia species	V
Klebsiella species*	V
Enterobacter species*	V

Technique
The medium is inoculated with the organism and incubated for 24 hours at 35°C.

Results after 24 hours:
Purple colour – positive reaction
Yellow colour – negative reaction

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Edwardsiella tarda ATCC® 15947

Negative control:
Citrobacter freundii ATCC® 8090

Precautions
Use light inocula for the tubes of lysine medium. Note the negative reaction of *Salmonella paratyphi A*. Do not read the test under 24 hours incubation. Some organisms may require prolonged incubation, up to 4 days.

References

1 Taylor W. I. (1961) *Appl. Microbiol. 9. 487–490.*
2 Falkow S. (1958) *Amer. J. Clin. Path. 29. 598–600.*
3 Moller V. (1955) *Acta Path. Microbiol. Scand. 36. 158–172.*

LYSINE IRON AGAR

Code: CM381

A diagnostic medium for salmonellae including S. arizona.

Formula	gm/litre
Bacteriological peptone	5.0
Yeast extract	3.0
Glucose	1.0
L-lysine	10.0
Ferric ammonium citrate	0.5
Sodium thiosulphate	0.04
Bromocresol purple	0.02
Agar	14.5
pH 6.7 ± 0.2	

Directions
Suspend 34 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Dispense into tubes and sterilise by autoclaving at 121°C for 15 minutes. Cool the tubes in an inclined position to form slants with deep butts.

Description
Lysine Iron Agar is a differential medium which detects salmonellae (including lactose fermenting *S. arizona*) by lysine decarboxylase activity and H_2S production. Edwards & Fife[1] developed the medium to detect lactose-fermenting salmonellae which will produce pink colonies on lactose-containing media e.g. DCA and BGA. In the usual examination for enteric pathogens these organisms would be overlooked. Further, many of these cultures, when transferred to Triple Sugar Iron (TSI) Agar slants, produced acid conditions in the medium so quickly that the expected positive reaction for hydrogen sulphide was suppressed. Since *S. arizona* strains which ferment lactose rapidly are found occasionally in outbreaks of food infection, it is important to determine their occurrence.

The only recognised groups of Enterobacteriaceae which regularly decarboxylate lysine rapidly and which produce large amounts of hydrogen sulphide, are the salmonellae[2,3].

Lysine Iron Agar is therefore a sensitive medium for the detection of lactose-fermenting and non lactose-fermenting salmonellae.

Technique
The medium is tubed, sterilised and slanted so that a short slant and deep butt are formed. It is inoculated with a straight needle by stabbing to the base of the butt and streaking the slant. The caps of the tubes must be replaced loosely so that aerobic conditions prevail on the slant. Incubate at 35°C overnight.

Cultures which rapidly produce lysine decarboxylase cause an alkaline reaction (purple colour) throughout the medium. Those organisms that do not decarboxylate lysine produce an alkaline slant and an acid butt (yellow colour).

Cultures which produce hydrogen sulphide cause an intense blackening in the medium.

Due to deamination of the lysine, *Proteus* and *Providence* cultures produce a red slant over an acid butt.

Reactions

Cultures	*Slant*	*Butt*	H_2S
Salmonella	Alkaline	Alkaline	+
Proteus	Red	Acid	-
Providence	Red	Acid	-
Citrobacter	Alkaline	Acid	+
Escherichia	Alkaline	Acid or neutral	-
Shigella	Alkaline	Acid	-
Klebsiella	Alkaline	Alkaline	-

Thatcher & Clark[4] described a procedure for the isolation of salmonellae from foods in which suspect colonies from selective agar plates were purified and then inoculated into Lysine Iron Agar and Triple Sugar Iron Agar. Using this combination of media a greater discrimination can be made between the coliform organisms, e.g. *Escherichia* and *Shigella.*

Timms[5] described the techniques of isolation and identification of salmonellae infection in turkeys, using Lysine Iron Agar.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Enterobacter aerogenes ATCC® 13048
lysine decarboxylation

Proteus mirabilis NCTC 10975
deamination

Negative control:
Enterobacter cloacae ATCC® 23355

Precautions
Salmonella paratyphi A does not produce lysine decarboxylase and therefore will give an alkaline slant and an acid butt.

H_2S-producing *Proteus* species do not blacken this medium[6].

References

1 Edwards P. R. and Fife Mary A. (1961) *Appl. Microbiol. 9. 478–480.*
2 Moeller V. (1954) *Acta. Pathol. Microbiol. Scand. 355. 259–277.*
3 Ewing W. H., Davis B. R. and Edwards P. R. (1960) *Pub. Hlth Labs. 18. 77–83.*
4 Thatcher F. S. and Clark D. S. (1968) *University of Toronto Press, p. 100.*
5 Timms L. (1971) *Med. Lab. Tachn. 28. 150–156.*
6 Finegold S. M. & Martin W. J. (1982) *Bailey & Scott's Diagnostic Microbiology. 6th Edn. C. V. Mosby. St. Louis. p.631.*

LYSINE MEDIUM

Code: CM191

A synthetic medium for the isolation and enumeration of wild yeasts encountered in brewing. On this medium, pitching yeasts are suppressed.

Formula	gm/litre
Glucose	44.5
Potassium dihydrogen phosphate	1.78
Magnesium sulphate	0.89
Calcium chloride fused	0.178
Sodium chloride	0.089
Adenine	0.00178
DL-methionine	0.000891
L-histidine	0.000891
DL-tryptophane	0.000891
Boric acid	0.0000089
Zinc sulphate	0.0000356
Ammonium molybdate	0.0000178
Manganese sulphate	0.0000356
Ferrous sulphate	0.0002225
Lysine	1.0
Inositol	0.02
Calcium pantothenate	0.002
Aneurine	0.0004
Pyridoxine	0.0004
p-aminobenzoic acid	0.0002
Nicotinic acid	0.0004
Riboflavin	0.0002
Biotin	0.000002
Folic acid	0.000001
Agar	17.8
pH (see directions)	

Directions
Suspend 6.6g in 100ml distilled water containing 1.0ml Potassium lactate 50% SR37. Bring to the boil to dissolve completely. Agitate frequently to prevent superheating. Cool to 50°C and add 0.1ml of lactic acid 10% SR21 to adjust to pH 4.8 $\pm$ 0.2. Dispense into petri dishes and remove surface moisture by drying at 37°C.

Description
A complex medium, originally described by Morris & Eddy[1] for the isolation and enumeration of wild yeasts in pitching yeast. Walters and Thiselton[2] examined 180 species of yeasts in a liquid synthetic medium containing lysine as the sole nitrogen source. They found that no normal *cerevisiae* or *carlsbergensis* strains utilised lysine whereas many other yeasts, including wild yeasts, did so. They kept their stock cultures on malt extract agar slopes or on malt extract chalk agar in the case of *Brettanomyces* species. Later, Morris & Eddy[1] described a solid lysine medium for the isolation and enumeration of wild yeasts in pitching yeast. Oxoid Lysine Agar is made to their published formula.

Technique
Wash and centrifuge the sample of pitching yeast three times with distilled water. Remove 0.2ml of a suspension containing approximately 10^7 cells per ml and spread with a bent platinum wire, over the surface of a Lysine Medium plate. Incubate at 25°C and examine daily for evidence of growth. Count the number of colonies which develop, and express the degree of contamination as the number of wild yeast cells per million cells of the original inoculum.

The number of cells in the inoculum is important as it has been shown by Morris & Eddy that small numbers of cells (approximately 100 to 1,000) still grow to a limited extent on the medium. Where the number of brewing yeast cells exceeds approximately 10,000, a count of the colonies developing provides a direct measure of the contamination by wild yeasts[3].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Pichia fermentans ATCC® 10651

Negative control:
Saccharomyces (carlsbergensis) uvarum ATCC® 2700

Precautions
The pitching yeast may grow as a slight background film with the 'wild' yeast appearing as colonies on the film.

References

1 Morris E. O. and Eddy A. A. (1957) *J. Inst. Brew. 63(1) 34–35.*
2 Walters L. S. and Thiselton M. R. (1953) *J. Inst. Brew. 59. 401.*
3 Fowell R. R. (1965) *J. Appl. Bact. 28. 373–383.*

M17 AGAR

Code: CM785

For improved growth of lactic streptococci and their bacteriophages and selective enumeration of Streptococcus thermophilus from yogurt.

Formula	gm/litre
Tryptone	5.0
Soya peptone	5.0
'Lab-Lemco' powder	5.0
Yeast extract	2.5
Ascorbic acid	0.5
Magnesium sulphate	0.25
Di-sodium-glycerophosphate	19.0
Agar	11.0
pH 6.9 ± 0.2	

Directions
Suspend 48.25g in 950ml of distilled water and bring gently to the boil. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add 50ml of sterile lactose solution (10% w/v).

Lactose solution 10% (w/v)
Dissolve 10g of Lactose Code L70 in 100ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes or by membrane filtration through a 0.2µm membrane.

Description
M17 Agar CM785 is based on the formulation described by Terzaghi and Sandine[1] and is recommended as an improved medium for the growth and enumeration of lactic streptococci and their bacteriophages.

Because it supports better host growth it allows the demonstration of phenomena commonly associated with other bacterial virus systems but not previously reported for lactic streptococcal phages and makes possible detailed studies of plaque morphology and lysogeny.

Lactic streptococci are nutritionally fastidious and require complex media for optimal growth[2,3]. Their homofermentative acid-producing nature requires that the medium is well buffered so that the culture pH is maintained above 5.7 during active growth. This maintenance of the pH is important as lower pH can result in injury and reduced recovery of Lactic streptococci.

M17 Agar contains di-sodium-glycerophosphate which has sufficient buffering capacity to maintain the pH above 5.7 of actively growing cultures even after 24 hours at 30°C. This buffering agent also allows the addition of calcium without a precipitation complex being formed. The calcium-containing medium is used for the assay of bacteriophages of Lactic streptococci[1].

Shankar and Davies[4] reported that M17 Agar was suitable for the isolation and enumeration of *Streptococcus thermophilus* from yogurt as the high concentration of di-sodium-glycerophosphate resulted in suppression of *Lactobacillus bulgaricus*. M17 Agar has been recommended[5,6] by the International Dairy Federation for the selective enumeration of *Streptococcus thermophilus* from yogurt.

M17 Agar is also suitable for growing and maintaining starter cultures for cheese and yogurt manufacture as it has little deleterious effect on their subsequent acid-producing ability in milk at either 30°C or 22°C[1].

One further useful property of this agar is its ability to detect streptococcal mutants which are unable to ferment lactose[1]. These mutant Lac- strains form much smaller colonies than the parent lactose fermenting strain.

Technique
Bacteriophage assay.
Microbiologists wishing to assay phage activity should consult the paper of Terzaghi and Sandine[1] for a comprehensive description of the method.

For the enumeration of Streptococcus thermophilus in yogurt.
1 Mix or blend the yogurt sample to obtain a uniform homogenicity.

2 Weigh 10 ± 0.1 grams of the test sample into a 200ml round bottom centrifuge tube made of strengthened glass, or the container of the mechanical mixer.

3 Add sterile 0.1% (w/v) peptone solution* to the test sample until the mass of the test sample and diluent is 50 grams.

4 Prepare a suitable series of decimal dilutions of the yogurt suspension in 9ml volumes of sterile 0.1% (w/v) peptone solution.

5 (i) Inoculate duplicate plates of M17 Agar with a loopful from each dilution and spread to obtain single colonies.

(ii) Add duplicate 1ml aliquots of the dilution into a petri dish and prepare pour plates with 14ml of sterile M17 Agar cooled to 43°C ± 1°C.

6 Incubate at 35°C for 48 hours.

7 Examine the plates after 24 and 48 hours incubation.

Streptococcus thermophilus colonies are visible after 18–24 hours and after 48 hours incubation form colonies of 1–2mm in diameter.

Lactobacillus bulgaricus do not grow or produce very restricted colonies.

8 Carry out the counts on pour plates and express the results as the number of colony forming units per gram of sample.

0.1% peptone water[6] can be prepared as follows:*

L42	Tryptone	0.5 g
L49	Peptone P	0.5 g
	Distilled water	1 litre

Sterilise by autoclaving at 121°C for 15 minutes.

Confirmation
Colonies isolated from milk products that are suspected to be *Streptococcus thermophilus* can be confirmed by the Gram stain (Gram positive cocci) and catalase test (negative).

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Streptococcus thermophilus ATCC® 14485

Negative control:
Lactobacillus bulgaricus ATCC® 11842

References

1 Terzaghi B. E. and Sandine W. E. (1975) *Applied Microbiology 29. 807–813.*

2 Anderson A. W. and Elliker P. R. (1953) *J. Dairy Science 36. 161–167.*

3 Reiter B. and Oram J. D. (1962) *J. Dairy Res. 29. 63–77.*

4 Shankar P. A. and Davies F. L. (1977) *J. Soc. Dairy Technology 30. 28–30.*

5 International Dairy Federation (1981) *Joint IDF/ISO/AOAC Group E44.*

6 International Organization for Standardization (1985) *ISO/DIS 7889.*

M17 BROTH

Code: CM817

For improved growth of lactic streptococci and their bacteriophages.

Formula	gm/litre
Tryptone	5.0
Soya peptone	5.0
'Lab-Lemco' powder	5.0
Yeast extract	2.5
Ascorbic acid	0.5
Magnesium sulphate	0.25
Di-sodium-glycerophosphate	19.0
pH 6.9 ± 0.2	

Directions
Suspend 37.25g in 950ml of distilled water and bring gently to the boil. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add 50ml of sterile lactose solution (10% w/v).

Lactose solution 10% (w/v)
Dissolve 10g of Lactose Code L70 in 100ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes or by membrane filtration through a 0.2mm membrane.

Description
M17 Broth CM817 has been produced in parallel with M17 Agar CM785. Its use in conjunction with M17 Agar in bacteriophage assays has been described by Terzaghi and Sandine[1]. These workers also suggest that M17 Broth would be a suitable medium for the maintenance of starter cultures because of its considerable buffering capacity and the little effect it has on the subsequent acid-producing ability of these cultures.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Streptococcus thermophilus ATCC® 14485

Negative control:
Lactobacillus bulgaricus ATCC® 11842

References

1 Terzaghi B. E. and Sandine W. E. (1975) *Applied Microbiology 29. 807–813.*

2 Anderson A. W. and Elliker P. R. (1953) *J. Dairy Science 36. 161–167.*

3 Reiter B. and Oram J. D. (1962) *J. Dairy Res. 29. 63–77.*

4 Shankar P. A. and Davies F. L. (1977) *J. Soc. Dairy Technology 30. 28–30.*

5 International Dairy Federation (1981) *Joint IDF/ISO/AOAC Group E44.*

6 International Organization for Standardization (1985) *ISO/DIS 7889.*

MACCONKEY AGAR

Code: CM7

A differential medium for the isolation of coliforms and intestinal pathogens in water, dairy products and biological specimens.

Formula	gm/litre
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.075
Agar	12.0
pH 7.4 $\pm$ 0.2	

Directions
Suspend 52g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Dry the surface of the gel before inoculation.

Description
A differential medium for the detection, isolation and enumeration of coliforms and intestinal pathogens in water, dairy products and biological specimens. MacConkey Agar CM7 corresponds to the medium recommended by the World Health Organization[1], the Dept. of Health[2] and by Windle Taylor [3] for the bacteriological examination of water.

Although principally used for coliforms, this medium may also be employed for the differentiation of other enteric bacteria (including pathogens) and is suitable for the differentiation of *Pasteurella* species[4].

Technique
Pathological specimens
Due to its ability to support the growth of pathogenic Gram-positive cocci (e.g. staphylococci and enterococci) as well as Enterobacteriaceae, MacConkey Agar CM7 is particularly recommended for the cultivation of pathogens which may be present in a variety of specimens such as urine, faeces and wound swabs. Whilst it is selective it does not suppress a mixed bacterial flora to the same extent as other inhibitory media (including other MacConkey agars). It provides a number of other diagnostic indications in addition to bile tolerance, such as colony morphology and chromogenesis. MacConkey Agar should be used in parallel with other selective indicator media such as Desoxycholate Citrate Agar, Bismuth Sulphite Agar, Brilliant Green Agar and Brilliant Green Bile (2%) Broth, and a non-selective medium such as Blood Agar.

Water Examination[2,3]
The medium may be used for the direct count of coli-aerogenes bacteria, using pour-plates prepared from known volumes of the water sample, but a more exact role for the medium is for the differentiation of organisms producing acid and gas in MacConkey Broth at 35°C: all positive broth tubes are plated on MacConkey Agar, the plates are incubated for 24 hours at 35°C and examined for typical colonies (see below). Colonies composed of Gram-negative non-sporing rods are subcultured for further identification.

The presence of enterococci in azide or tellurite media may be confirmed by subculture on MacConkey Agar. See below for colonial morphology.

Yersinia and Pasteurella differentiation
MacConkey Agar can be used to differentiate *Yersinia* species from *Pasteurella* species[4]. *Yersinia pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica* will show growth on MacConkey Agar after 24 hours incubation at 35°C[5]. *Pasteurella* species (including *P. multocida*) will not grow on MacConkey Agar.

Pectinolytic Organisms (Stewart[6])
Stewart used Oxoid MacConkey Agar as the basis of a selective-diagnostic medium for pectinolytic organisms, in order to isolate soft-rot *Erwinia* species from specimens containing other Enterobacteriaceae. MacConkey Agar-calcium chloride plates (5.2g CM7 powder, 0.4g $CaCl_2$, 75ml distilled water) overlaid with a pectate-EDTA layer (0.1% EDTA containing 2% sodium polypectate) are inoculated and incubated for 48 hours at 25°C. Lactose fermenting *Erwinia* produce red colonies in shallow pits formed by pectate liquefaction.

Colonial Characteristics
After 24 hours at 35°C typical colonies are as follows:

Organism	***Colour***	***Remarks***
Escherichia coli	red	non-mucoid
Aerobacter aerogenes	pink	mucoid
Enterococcus species	red	minute, round
Staphylococci	pale pink	opaque
Pseudomonas aeruginosa	green-brown	fluorescent growth

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium at 2–8°C.

Quality Control
Positive control:
Enterococcus faecalis ATCC® 29212
Staphylococcus aureus ATCC® 25923

Negative control:
Uninoculated medium

Precautions
The colonial characteristics described give presumptive identification only of the isolated organisms. It is necessary to subculture and carry out confirmation tests for final identification.

To enhance the pigment of suspected *Staphylococcus aureus*, hold the plates on the bench at ambient temperature for 12–l8 hours.

References

1 World Health Organization (1963) *International Standards for Drinking Water 2nd ed. WHO, Geneva.*
2 Departments of the Environment, Health, Social Security and Public Health Laboratory Service (1982) *The Bacteriological Examination of Drinking Water Supplies. Report No.71. HMSO. London.*

3 Windle Taylor E. (1958) *'The examination of Waters and Water Supplies' 7th ed., Churchill Ltd., London.*
4 Hoogendijk J. L. (1962) *Antonie van Leeuwenhoek J. Microbiol. Serol. 28(3) 315–320.*
5 Wilson G. S. and Miles A. A. (1964) *'Topley and Wilson's Principles of Bacteriology and Immunity' 5th Ed., Edward Arnold Ltd., London. vol.2.*
6 Stewart D. J. (1962) *Nature 195(4845), 1023.*

MACCONKEY AGAR

(WITHOUT SALT)

Code: CM7b

A differential medium on which swarming of Proteus species is suppressed. Recommended for urine examination.

Formula	gm/litre
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Neutral red	0.075
Agar	12.0
pH 7.4 ± 0.2	

Directions
Suspend 47g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring. Dry the surface of the gel before inoculation.

Description
This medium has the same formulation as MacConkey Agar CM7 except that it does not contain added salt and therefore provides a 'low electrolyte medium' on which most *Proteus* species do not spread. For this reason the medium has found particular favour for use in the examination of urine so that overgrowth of other organisms is prevented.

MACCONKEY AGAR NO.2

Code: CM109

A modification of MacConkey Agar No.3 containing Oxoid Bile Salts No.2 for the recognition of enterococci.

Formula	gm/litre
Peptone	20.0
Lactose	10.0
Bile salts No.2	1.5
Sodium chloride	5.0
Neutral red	0.05
Crystal violet	0.001
Agar	15.0
pH 7.2 ± 0.2	

Directions
Suspend 51.5g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
MacConkey Agar No.2 is a modification of the original MacConkey solid medium and is especially useful for the recognition of enterococci, in the presence of coliforms and non-lactose fermenters from water, sewage, food products, etc.

On this medium enterococci appear as small intensely red colonies with a pale periphery about 1mm in diameter. These organisms are frequently sought as an index of faecal pollution. Non-lactose fermenters are colourless. Bile tolerant micrococci, such as staphylococci and non-faecal streptococci, are completely inhibited.

McGeachie & Kennedy[1] employed Oxoid MacConkey Agar No.2 in a simplified method for counting the bacteria in urine. Using a bacteriological loop (delivering a known volume) they streaked well mixed uncentrifuged urine directly on to a blood agar and a MacConkey Agar plate – and spread the urine in a 1cm wide strip across one edge of the plate using 20 strokes. With a second sterile loop they spread a 1cm wide portion to form a second strip at right angles to the first. This was repeated to give a square pattern of four 1cm wide strips around the edge of the plate. After incubation, growth was noted as +, + +, + + +, or + + + + depending on whether 1, 2, 3 or 4 sides of the square showed colonies. The approximate estimate obtained agreed well with a more complicated pour-plate method and the simplified method was recommended for routine use.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control
Positive control:
Enterococcus faecalis ATCC® 29212

Negative control:
Uninoculated medium

Reference
1 McGeachie J. and Kennedy A. C. (1963) *J. Clin. Path. 16. 32–38.*

MACCONKEY AGAR NO.3

Code: CM115

A selective medium giving excellent differentiation between coliforms and non-lactose fermenters with inhibition of Gram-positive micrococci.

Formula	gm/litre
Peptone	20.0
Lactose	10.0
Bile salts No.3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0
pH 7.1 ± 0.2	

Directions
Suspend 51.5g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A more selective modification of MacConkey medium which is suitable for the detection and enumeration of coliform organisms and also for the detection and isolation of *Salmonella* and *Shigella* species occurring in pathological and food specimens. Due to the inclusion of a specially prepared fraction of bile salts in addition to crystal violet, the medium gives improved differentiation between coliforms and non-lactose fermenting organisms whilst Gram-positive cocci are completely inhibited.

This formulation corresponds with that recommended by the American Public Health Association[1] for the direct plating of water samples for coliform bacilli, for the examination of food samples for food poisoning organisms[2] and for the isolation of *Salmonella* and *Shigella* species in cheese[3].

Amongst other examples of the use of Oxoid MacConkey Agar No.3 are: the count of coli-aerogenes bacteria in poultry faecal specimens[4]; the count of coli-aerogenes bacteria in cattle and sheep faeces[5]; the count of coli-aerogenes and non-lactose fermenting organisms in poultry carcases[6]; bacterial counts on irradiated canned minced chicken[7]; the recognition of coli-aerogenes bacteria during investigations on the genus *Aeromonas*[8].

Anderson et al.[9] added 10µg/ml of kanamycin to MacConkey Agar to isolate epidemic strains of *Citrobacter diversus* which were causing neonatal meningitis.

The addition of 100mg of 4-methylumbelliferyl-β-D-glucuronide to one litre of MacConkey Agar detects the enzyme β-glucuronidase[10]. The cleaved 4-methylumbelliferyl moiety is fluorescent at 366nm. Thus colonies of *Esch. coli* can be detected rapidly in mixed cultures by examining the plate under a UV lamp after overnight incubation at 35°C. However, it should be remembered that other organisms may also be β-glucuronidase positive.

Technique
After inoculation the plates are usually incubated for 18 to 24 hours at 35°C and for a further 24 hours if non-lactose fermenting organisms are sought and have not appeared. Lower incubation temperatures may sometimes be used for more psychrophilic species. After 18 hours at 35°C, coliforms produce intense violet-red colonies whilst non-lactose fermenters are colourless.

Storage conditions and Shelf life
The dehydrated medium should be stored below 25°C and used before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Escherichia coli ATCC® 25922
Shigella sonnei ATCC® 25931

Negative control:
Enterococcus faecalis ATCC® 29212

Precautions
Prolonged incubation may lead to confusing results. Do not incubate beyond 48 hours.

Test the medium with a laboratory stock strain of *Shigella* species which is in the R-phase. R-phase shigellae should grow satisfactorily on MacConkey Agar.

References

1 American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington DC.*
2 American Public Health Association (1976) *Compendium of methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.*
3 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.*
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5 Medrek T. F. and Barnes Ella M. (1962) *J. Appl Bact. 25(2). 159–168.*
6 Barnes Ella M. and Shrimpton D. H. (1957) *J. Appl. Bact. 20(2). 273–285.*
7 Thornley Margaret J. (1957) *J. Appl. Bact. 20(2). 273–285.*
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10 Trepeta A. W. and Edburg S. C. (1984) *J. Clin. Microbiol. 19. 172–174.*
11 Maddocks J. L. and Greenan M. J. (1975) *J. Clin. Pathol. 28. 686–687.*

SORBITOL MACCONKEY AGAR

Code: CM813

A selective and differential medium for the detection of Escherichia coli O157.

Formula	gm/litre
Peptone	20.0
Sorbitol	10.0
Bile salts No.3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0
pH 7.1 ± 0.2	

Directions
Suspend 51.5g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Sorbitol MacConkey Agar is based on the formulation described by Rappaport and Henig[1], and is recommended for the isolation of pathogenic *Esch. coli* O157. The formulation is identical to MacConkey Agar No.3 except that lactose has been replaced with sorbitol. *Esch. coli* O157 does not ferment sorbitol and therefore produces colourless colonies. In contrast, most *Esch. coli* strains ferment sorbitol and form pink colonies. The efficiency of Sorbitol MacConkey Agar has been confirmed by March and Ratnam[2]. These workers reported that the detection of *Esch. coli* O157 on this medium had a sensitivity of 100% and a specificity of 85%. They recommended the medium as

a simple, inexpensive, rapid and reliable means of screening *Esch. coli* O157.

Esch. coli O157 has recently been recognised as a cause of haemorrhagic colitis, an illness characterised by bloody diarrhoea and severe abdominal pain. There is mounting evidence linking *Esch. coli* O157 and haemorrhagic colitis with haemolytic uraemic syndrome (HUS)[3,4,5,6,7].

Technique
1 Make up the agar according to the directions and pour into petri dishes. If necessary dry the surface of the agar.
2 Inoculate the plates with a suspension of the food, faeces, etc. to produce separated colonies.
3 Incubate at 35°C for 24 hours. Doyle and Schoeni[8] have reported that 35–37°C is the optimal temperature for growth of *Esch. coli* O157. At 44 to 45.5°C this *Esch. coli* serotype does not grow well even after 48 hours incubation.

Delay in reading plates beyond 24 hours should be avoided because the colour intensity of sorbitol-fermenting colonies fades, reducing the contrast with non-fermenting colonies.

Other Gram negative organisms including *Pseudomonas*, *Proteus* and *Klebsiella* species are able to grow on Sorbitol MacConkey Agar but may generally be differentiated by the appearance of their colonies.

A diagnostic reagent *Escherichia coli* O157 latex test DR620 is available so that instant confirmatory tests can be made from suspicious colonies.

Colonial Morphology
Esch. coli O157 will form colourless but otherwise typical *Esch. coli* colonies.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control
Positive control:
Escherichia coli O157

Negative control:
Escherichia coli ATCC® 25922

Precautions
Although the great majority of *Esch. coli* O157 strains have a typical appearance on Sorbitol MacConkey Agar, some strains are atypical[9].

Sorbitol MacConkey Agar cannot be used solely to detect VTEC strains of *Esch. coli* as some non-toxic strains will not ferment sorbitol[10].

References

1 Rappaport F. and Henig E. (1952) *J. Clin. Path. 5. 361.*
2 March S. B. and Ratnam S. (1986) *J. Clin. Microbiol. 23. 869–872.*
3 Centers for Disease Control 1985 – *United States, 1984, Morbid Mortal Weekly Rep., 34. 20–21.*
4 Karmali M.A., Petric M., Lim C., Fleming P. C., Arbus G. S. and Lior H. (1985) *J. Infect. Dis. 151. 775–782.*
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7 Waters J. R. (1985) *Can. Dis. Weekly Rep. 11. 123–124.*
8 Doyle M. P. and Schoeni S. L. (1984) *Appl. and Envir. Microbiol. 48. 855–856.*
9 Karmali M. A. (1988) *Culture 9. 2.*
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CEFIXIME-TELLURITE SUPPLEMENT

Code: SR172

A freeze-dried supplement for use with Sorbitol MacConkey Agar, CM813, for the selective isolation of E. coli O157:H7.

Vial contents	Milligrams	Mg/litre
Potassium tellurite	1.25	2.5
Cefixime	0.025	0.05

Directions
Aseptically add 2ml of sterile distilled water to 1 vial of Cefixime-Tellurite Supplement SR172E. Mix gently to dissolve the contents completely.

Add the vial contents to 500ml of Sorbitol MacConkey Agar prepared as directed and cooled to 50°C. Mix well and pour the medium into petri dishes.

Description
Chapman and co-workers[1], added cefixime and potassium tellurite to Sorbitol MacConkey Agar to improve the selectivity of the medium. The level of potassium tellurite selects serogroup O157 from other *E. coli* serogroups and inhibits *Providencia* spp. and *Aeromonas* spp. Cefixime is inhibitory to *Proteus* spp.

The use of cefixime and tellurite in Sorbitol MacConkey Agar for isolation of *E. coli* O157:H7 is described in the FDA Bacteriological Analytical Manual[2].

Storage conditions and Shelf life
Cefixime-Tellurite Supplement SR172 should be stored in the dark at temperatures below 0°C.

Prepared medium may be stored for up to 2 weeks in plastic bags.

Quality Control

Positive control:	
E. coli O157	NCTC 12079
Negative control:	
E. coli	ATCC® 25922

References

1 Zadik P.M., Chapman P.A. and Siddons C.A. (1993) *J. Med. Microbiol. 39. 155–158.*
2 Food and Drug Administration (1995) *Bacteriological Analytical Manual. 8th Edition. AOAC International. Gaitherburg. MD. Chapter 4, 20–23.*

MACCONKEY BROTH

Code: CM5

A differential medium containing neutral red for the detection of coliform organisms in water and milk examination.

Formula	gm/litre
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.075
pH 7.4 ± 0.2	

Directions
To prepare single strength broth, add 40g to 1 litre of distilled water. Mix well and distribute into containers fitted with fermentation (Durham) tubes. Sterilise by autoclaving at 121°C for 15 minutes.

Description
For the past fifty years, MacConkey Broth has been the standard medium for the primary isolation of coliform bacteria, and is still officially recommended for this purpose by the Public Health Laboratory Service Water Committee[7] and the World Health Organization[1]. The Oxoid product conforms to their specification for water testing and also to the formulation specified by the Dept. of Health[2] for milk grading.

The advantages of MacConkey Broth in the presumptive coliform test are the low proportion of false positive reactions (PHLS Water Subcommittee[3]) and the fact that most strains of *Escherichia coli* produce a positive reaction within 24 hours[4]. Disadvantages, due to variability of the peptone and bile salts contained in the original medium, have been overcome by large scale production, pooling of batches and careful quality control – including titrimetric standardisation of the bile salts by a method described by Burman[5].

The neutral red is pre-tested for the absence of toxic substances before inclusion in the Oxoid medium. Childs & Allen[6] have shown that some samples of neutral red were inhibitory. For those who prefer, this medium is also available with bromocresol purple as the indicator – for details of this alternative medium and the presumptive coliform test see MacConkey Broth (Purple) CM5a.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Escherichia coli (Turbidity + Gas) ATCC® 25922

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions
The neutral red indicator is carefully selected for this formulation and therefore shows no inhibitory effect. However, the more sensitive reaction of bromocresol purple in MacConkey Broth (Purple) CM5a is often preferred.

References

1 World Health Organization (1963) *International Standards for Drinking Water 2nd ed., WHO, Geneva.*
2 Dept. of Health (1937) *Memo 139/Foods, HMSO, London.*
3 Public Health Laboratory Service Water Subcommitee (1953) *J. Hyg. Camb. 51. 268–277.*
4 Windle Taylor E. (1958) *'The Examination of Waters and Water Supplies' 7th ed., Churchill Ltd., London.*
5 Burman N. P. (1955) *Proc. Soc. Water Treat. Exam. 4. 10–20 and discussion 20–26.*
6 Childs Eileen and Allen L. A. (1953) *J. Hyg. Camb. 51. 468–477.*
7 Dept. of Health and Social Security (1969) *4th impression, HMSO London.*

MACCONKEY BROTH (PURPLE)

Code: CM5a (Powder)
Code: CM6a (Tablets)

A differential medium containing BCP for the detection of coliform organisms in water and milk examination.

Formula	gm/litre
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Bromocresol purple	0.01
pH 7.4 ± 0.2	

Directions
Powder To prepare single strength broth add 40g to 1 litre of distilled water. Distribute into containers fitted with fermentation (Durham) tubes. Sterilise by autoclaving at 121°C for 15 minutes.

Tablets Add 1 tablet to 10ml of distilled water. Insert a fermentation (Durham) tube and sterilise by autoclaving at 121°C for 15 minutes.

Description
MacConkey Broth has long been used as a presumptive medium for the detection of the coli-aerogenes organisms. In the original medium, litmus was employed as the indicator of acid production but, in later publications, MacConkey suggested neutral red as a more satisfactory alternative. Childs & Allen[1] showed that some samples of neutral red exerted an inhibitory effect on the growth of *Escherichia coli* in this medium.

Bromocresol purple is less inhibitory, and the colour change from purple to yellow provides a more sensitive and definite indication of acid formation; therefore this indicator is used in Oxoid MacConkey Broth (Purple), which corresponds to the alternative formulations recommended in 'The Bacteriological Examination of Water Supplies'[2] and 'International Standards for Drinking Water'[3].

Technique
The presumptive coliform examination consists of the inoculation of measured volumes of water into tubes of MacConkey Broth (Purple) which are incubated at

35°C for 48 hours. Choice of volumes for inoculation will depend on the bacteriological grade of the water being tested; for 'medium' waters the Public Health Laboratory Service Water Committee (1961) recommend one 50ml, five 10ml and five 1ml quantities of water – 50ml and 10ml amounts being added to their own volume of double-strength MacConkey Broth while the 1ml amounts are each added to 5ml of single-strength MacConkey Broth. Acid formation is indicated by a yellow colouration of the broth, and gas formation is indicated by an amount of gas at least sufficient to fill the concavity at the top of the Durham tube. From the number of tubes showing the presence of acid and gas, the most probable number of (presumed) coliform bacteria present in 100ml of the original water may be estimated by reference to probability tables; these tables based on McCrady's computations, are included in Report No.71: 'The Bacteriological Examination of Water Supplies'[2] and in many other publications dealing with this subject. For the differential coliform test, each MacConkey tube showing acid and gas is then subcultured into a fresh tube of MacConkey Broth and incubated at 44°C. Formation of gas within 48 hours is practically specific for *Escherichia coli* and indicative of faecal pollution of the original water sample.

MacConkey Broth (Purple) is also suitable for the bacteriological examination of milk, as described by Davis[4]. This method, which is basically similar to that used for the examination of water, consisting in the inoculation of suitable dilutions of the milk into tubes of this medium followed by incubation and inspection, was originally recommended by the Dept. of Health, London[5].

MUG Reagent BR71 – The addition of 4-methylumbelliferyl-β-D-glucuronide (MUG) BR71 to this medium will enhance the detection of *Escherichia coli.* See MUG Reagent BR71 under Biochemical Reagents for further details.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Quality Control
Positive control:
Escherichia coli (Acid + Gas) ATCC® 25922

Negative control:
Staphylococcus aureus ATCC® 25923

References

1 Childs Eileen and Allen L. A. (1953) *J. Hyg. Camb. 51(4). 468–477.*
2 Departments of the Environment, Health, Social Security and Public Health Laboratory Service (1982) *The Bacteriological Examination of Drinking Water Supplies. Report No. 71. HMSO London.*
3 World Health Organization (1963) *'International Standards for Drinking Water' 2nd ed., WHO, Geneva.*
4 Davis J. G. (1959) *'Milk Testing' 2nd ed., Dairy Industries Ltd., London.*
5 Dept. of Health (1937) *Memo. 139/Foods, HMSO, London.*

MALT EXTRACT AGAR

Code: CM59

A medium for the detection, isolation and enumeration of yeasts and moulds. Bacteria may be suppressed by the addition of lactic acid.

Formula	gm/litre
Malt extract	30.0
Mycological peptone	5.0
Agar	15.0
pH 5.4 ± 0.2	

Directions
Suspend 50 grams in 1 litre of distilled water and boil to dissolve. Sterilise by autoclaving at 115°C for 10 minutes.

If it is desired to adjust the reaction to pH 3.5, cool to 55°C and add 10% Lactic Acid SR21 to the Malt Extract Agar. Once acidified with lactic acid, the medium should not be re-heated.

Description
This medium, similar to the one described by Galloway & Burgess[1] is recommended for the detection, isolation and enumeration of yeasts and moulds. For mycological counts it may be desirable to prepare the more acid medium in order to suppress bacterial growth.

Also see Wort Agar.

MALT EXTRACT BROTH

Code: CM57

A liquid medium recommended for the cultivation of moulds and yeasts, especially during tests for sterility.

Formula	gm/litre
Malt extract	17.0
Mycological peptone	3.0
pH 5.4 ± 0.2	

Directions
Add 20g to 1 litre of distilled water. Mix well, distribute into final containers and sterilise by autoclaving at 115°C for 10 minutes. This liquid medium is recommended for the cultivation of moulds and yeasts, during tests for sterility, etc.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Aspergillus niger ATCC® 9642
Candida albicans ATCC®10231

Negative control:
Bacillus cereus (at pH 3.5) ATCC® 10876

Precautions
Avoid overheating as the acid pH will soften the agar in the presence of heat.

Reference
1 Galloway L. D. and Burgess R. (1952) *'Applied Mycology and Bacteriology' 3rd ed., Leonard Hill, London, pp. 54 and 57.*

MANNITOL SALT AGAR

Code: CM85

A selective medium for the isolation of presumptive pathogenic staphylococci. Most other bacteria are inhibited, with the exception of a few halophilic species.

Formula	gm/litre
'Lab-Lemco' powder	1.0
Peptone	10.0
Mannitol	10.0
Sodium chloride	75.0
Phenol red	0.025
Agar	15.0
pH 7.5 ± 0.2	

Directions
Suspend 111g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A selective medium prepared according to the recommendations of Chapman[1] for the isolation of presumptive pathogenic staphylococci. Most other bacteria are inhibited by the high salt concentration with the exception of some halophilic marine organisms. Presumptive coagulase-positive staphylococci produce colonies surrounded by bright yellow zones whilst non-pathogenic staphylococci produce colonies with reddish purple zones.

Mannitol Salt Agar is recommended for the detection and enumeration of coagulase-positive staphylococci in milk[2], in food[3] and other specimens[4].

Oxoid Mannitol Salt Agar has been used for the examination of meat or fish[5,6,7,8,9].

The addition of 5% v/v Egg Yolk Emulsion SR47 to Mannitol Salt Agar enables the lipase activity of staphylococci to be detected as well as mannitol fermentation[10]. The high concentration of salt in the medium clears the egg yolk emulsion and lipase production is detected as a yellow opaque zone around colonies of staphylococci which produce this enzyme.

Technique
Heavily inoculate the Mannitol Salt Agar plate and incubate for 36 hours at 35°C or for 3 days at 32°C – the latter is recommended by the APHA[3].

Presumptive coagulase-positive staphylococci produce colonies with bright yellow zones whilst coagulase-negative staphylococci are surrounded by a red or purple zone. Pick off suspect colonies and subculture in a medium not containing an excess of salt (e.g. Nutrient Broth No.2 CM67) to avoid interference with coagulase or other diagnostic tests.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923
Staphylococcus epidermidis ATCC® 12228

Negative control:
Escherichia coli ATCC® 25922

Precautions
A few strains of *Staph. aureus* may exhibit a delayed fermentation of mannitol. Negative plates should be re-incubated overnight before discarding.
Presumptive *Staph. aureus* must be confirmed with a coagulase test (Staphylase Test DR595).

References
1 Chapman G. H. (1945) *J. Bact. 50. 201–203.*
2 Davis J. G. (1959) *'Milk Testing' 2 ed., Dairy Industries Ltd., London.*
3 American Public Health Association (1966) *'Recommended Methods for the Microbiological Examination of Foods' 2nd Ed., APHA Inc., New York.*
4 Silverton R. E. and Anderson M. J. (1961) *'Handbook of Medical Laboratory Formulae' Butterworths, London.*
5 Barnes Ella M. and Shrimpton D. H. (1957) *J. Appl. Bact. 20. 273–285.*
6 Thornley Margeret J. (1957) *J. Appl. Bact. 20. 286–298.*
7 Bain Nora, Hodgkiss W. and Shewan J. M. (1958) *DSIR, Proc. 2nd Internat. Symp. Food Microbiol., 1957, HMSO, London, pp. 103–116.*
8 Spencer R. (1961) *J. Appl. Bact. 24. 4–11.*
9 Eddy B. P. and Ingram M. (1962) *J. Appl. Bact. 25. 237–247.*
10 Gunn B. A., Dunkelberg W. E. and Creitz J. R. (1972) *Am. J. Clin. Path. 57. 236–238.*

MAXIMUM RECOVERY DILUENT

(PEPTONE SALINE DILUENT)

Code: CM733

A protective and isotonic diluent for maximal recovery of micro-organisms (ISO/DIS 6649).

Formula	gm/litre
Peptone	1.0
Sodium chloride	8.5
pH 7.0 ± 0.2	

Directions
Dissolve 9.5g in 1 litre of distilled water. Dispense into the final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
Maximum Recovery Diluent CM733 combines the protective effect of peptone in the diluting solution[1] with the osmotic support of physiological saline.

The low concentration of peptone does not cause multiplication of the organisms within 1–2 hours of dilution of the sample.

The isotonic strength of the diluent ensures recovery of organisms from various sources which may be vulnerable in distilled water or aqueous suspensions.

Technique

1 Prepare the medium according to the directions. For the 150 method[3] distribute the medium into 90ml volumes or into 9ml volumes.
2 Put 10g of the test sample into a sterile blender jar or sterile plastic bag.
3 Add 90ml of sterile Maximum Recovery Diluent.
4 Operate the blender according to its speed for sufficient time to give a total number of 15,000 to 20,000 revolutions. Alternatively operate a peristaltic type blender (Stomacher) for 2 minutes.
5 Within 15 minutes transfer 1ml of the macerate to 9ml of sterile diluent and mix well (10^{-1} dilution).
6 Prepare additional decimal dilutions in the same way.
7 Aseptically transfer 1ml of each dilution of the initial suspension in duplicate to the centres of petri dishes.
8 Prepare pour plates with the medium of choice.
9 Allow the agar to solidify and incubate.

Storage conditions and Shelf life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control

Use a positive test sample divided between new and previous lot/batch of diluent. Carry out duplicate tests as described in Technique and look for equivalent yield of organisms between the diluent batches.

References

1 Straker R. P. and Stokes J. L. (1957) *Appl. Microbiol. 5. 21–25.*
2 Patterson J. W. and Cassells J. A. (1963) *J. Appl. Bact. 26. 493–497.*
3 ISO/DIS 6649. Meat and Meat Products–Detection and Enumeration of Clostridium perfringens.

MEMBRANE ENDO AGAR LES

Code: MM551

A membrane filtration medium requiring Basic Fuchsin for enumeration of coliform organisms in water, using a 2-stage enrichment technique.

Formula	gm/litre
Yeast extract	1.2
Tryptone	3.7
Peptone P	3.7
Tryptose	7.5
Lactose	9.4
Dipotassium phosphate	3.3
Monopotassium phosphate	1.0
Sodium chloride	3.7
Sodium desoxycholate	0.1
Sodium lauryl sulphate	0.05
Sodium sulphite	1.6
Agar	10.0
pH 7.2 $\pm$ 0.2	

Basic Fuchsin to be added at 0.8gm/litre.

BASIC FUCHSIN

Code: BR50

For use with Endo Agar Base CM479

For each litre of medium use 8ml of a 10% w/v solution of this dye dissolved in 95% ethyl alcohol.

Directions

Suspend 45 grams in 1 litre of distilled water. Add 8ml of a 10% w/v alcoholic solution of Basic Fuchsin. Heat gently with frequent agitation until the medium boils. DO NOT AUTOCLAVE. Cool to 45°C and dispense into 50–60mm dishes in 4ml volumes. For larger dishes use sufficient medium to give an equivalent depth (approx. 1.5mm).

Plates should be protected from light and may be stored for up to two weeks in the refrigerator.

WARNING

Basic Fuchsin is a potential carcinogen and care must be taken to avoid inhalation of the powdered dye and contamination of the skin.

Description

M-Endo Agar LES is prepared according to the Lawrence Experimental Station formulation of McCarthy, Delaney and Grasso[1] and used for the enumeration of coliform organisms in water[2].

The value of the membrane filter technique for the enumeration of coliform organisms in water lies in its greater reliability and precision when compared with the MPN multiple tube test[3].

McCarthy, Delaney and Grasso[1] have recommended a two-stage process of enrichment to provide a non-toxic environment for maximal resuscitation of the coliforms.

Calabrese and Bissonnette[4] found that supplementation of M-Endo medium with catalase and sodium pyruvate resulted in improved recovery of coliform bacteria from chlorinated sewage effluent.

Experiments carried out by Noble[5] indicated that sodium sulphite and Basic Fuchsin can be extremely detrimental to the stressed coliforms, reducing the total count.

Enrichment for a period of 2 hours $\pm$ 0.5 hours in single strength Lauryl Tryptose Broth CM451 will give adequate resuscitation to the stressed coliform organisms and provide the best assessment of the quality of the drinking water.

Enrichment is usually not necessary for the examination of non-potable waters and sewage effluents.

Selection of the sample volume is governed by the expected bacterial density. An ideal quantity will result in growth of more than 50 coliform colonies and less than 200 colonies of all types.

All organisms which produce a colony with a golden-green metallic sheen within 24 hours incubation are considered members of the coliform group. The sheen may cover the entire colony or be restricted to the central area or the periphery. The recommended depth of M-Endo Agar LES in plates restricts the

colony size and hence facilitates carrying out the colony count.

Technique
The water sample is filtered through a sterile membrane filter[6].

For the first stage of enrichment, place a sterile incubating pad in the upper half of a sterile petri dish and pipette onto this 2ml of Lauryl Tryptose Broth CM451. Aseptically place the filter membrane on to the incubating pad and incubate, without inverting the dish, for 1–1.5 hours at 35°C in an atmosphere having 100% humidity. Place petri dishes of M-Endo Agar LES in the incubator for the entire period so that they will be at the correct temperature when required for the second stage of enrichment. The first stage enrichment culture is removed from the incubator and the filter membrane is stripped aseptically from the incubating pad and transferred to the surface of the petri dish of M-Endo Agar LES. It is important that complete contact is made between the membrane and the agar surface. The plate is inverted and incubated for 22–24 hours at 35°C.

Alternatively, the membrane filter incubating pad can be placed inside the lid of the petri dish of M-Endo Agar LES and 2ml of Lauryl Tryptose Broth CM451 pipetted onto the pad. The filter membrane is placed face upwards on the pad and incubated for 1–1.5 hours at 35°C.

To carry out the second stage of enrichment, the first-stage enrichment is removed from the incubator and the filter membrane is stripped from the pad and placed face upwards on the surface of the M-Endo Agar LES medium. The incubating pad is left in the lid and the plates are incubated in the inverted position for 24 hours at 35°C.

If preferred the second stage only may be used. The prepared membrane filter is placed directly on the agar surface and incubated as described.

All the organisms which produce a colony with a golden-green metallic sheen within 24 hours incubation may be considered as presumptive coliforms.

Calculation of Coliform Density
Report the coliform density in terms of total coliform/100ml. Compute the count using those membrane filters with 20–80 coliform colonies and not more than 200 of all types per membrane.

$$\text{Total Coliform colonies/100ml} = \frac{\text{Coliform colonies} \times 100}{\text{ml of sample filtered}}$$

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium in the dark and at 2–8°C.

Precautions
Use care when handling basic fuchsin to avoid inhaling the powder and staining the skin.

References

1 McCarthy J.A., Delaney J.E., Grasso R.J. (1961) *'Measuring Coliforms in Water', Water and Sewage Works, 108. 238–243.*
2 American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater. 15th Edn, APHA Inc, Washington DC.*
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MEMBRANE LAURYL SULPHATE BROTH

Code: MM615

A replacement medium for Membrane Enriched Teepol Broth for the enumeration of coliform organisms and Escherichia coli in water.

Formula	gm/litre
Peptone	39.0
Yeast extract	6.0
Lactose	30.0
Phenol red	0.2
Sodium lauryl sulphate	1.0
pH 7.4 ± 0.2	

Directions
Dissolve 76.2 grams in 1 litre of distilled water. Distribute into final containers, e.g. 100ml screw cap bottles. Sterilise by steaming for 30 minutes on three consecutive days or by autoclaving at 121°C for 15 minutes.

Description
In formulating Membrane Enriched Teepol Broth, Burman[1] substituted Teepol in place of bile salts in the membrane filtration test medium used to detect coliform organisms in water. The use of Teepol in place of bile salts had been previously recommended by Jameson and Emberley[2] and its value was confirmed by other workers (Jebb[3] and Windle-Taylor[4,5]). It is essential to use one standard grade of Teepol and Teepol 610 (BDH Ltd.) has been recommended.

Burman[6] showed that resuscitation media are not required with Membrane Enriched Teepol Broth if a preliminary incubation is carried out at a lower temperature. Thus non-chlorinated organisms benefit from 4 hours incubation at 30°C, but chlorinated organisms require 6 hours incubation at 25°C.

Membrane Lauryl Sulphate Broth is similar to Membrane Enriched Teepol Broth except that the selective agent Teepol 610 has been replaced by sodium lauryl sulphate.

In 1976 the production of Teepol 610 ceased and studies were carried out to identify a suitable alternative selective agent that could be incorporated into the basal medium. As a result of this work it was

recommended[7] that Teepol 610 should be replaced by sodium lauryl sulphate (BDH No.44244) at a concentration of 0.1% w/v.

Membrane Lauryl Sulphate Broth CM615 has been recommended[7,8] as a standard medium for the enumeration of coliform organisms and *Escherichia coli* from water and sewage by the membrane filtration technique.

The Medium and method are fully described in The Bacteriological Examination of Water Supplies Report 71[9].

Technique
The coliform and *Esch. coli* count are made on separate volumes of water. The volumes should be chosen so that the number of colonies to be counted on the membrane lies between 10 and 100. If the water is suspected to contain less than 100 coliform organisms per 100ml, then a 100ml sample should be filtered.

The water samples are filtered through a sterile membrane filter (Report 71[9]) and the membrane filter is placed face upwards on an absorbent pad previously saturated with Membrane Lauryl Sulphate Broth. The pad and membrane filter should be incubated in a vapour-tight container to prevent evaporation.

Membranes to be incubated at 44°C should be placed in watertight heavy containers and immersed in a closely controlled water-bath.

Burman recommends the following incubation periods and temperatures:

Unchlorinated waters

Coliform organisms	4 hours at 30°C followed by 14 hours at 35°C.
Esch. coli	4 hours at 30°C followed by 14 hours at 44°C.

If rapid results are required, the membrane may be examined after a total incubation time of 12 hours. If no colonies are present, a nil count can be assumed. If small colonies of indeterminate colour are present, then the membranes must be returned to the incubator for the full period.

Presumptive coliform organisms
After incubation, yellow colonies from membranes incubated at 35°C should be sub-cultured to Lactose Peptone Water to confirm that they will produce gas at 35°C after 43 hours incubation.

Presumptive Esch. Coli
Yellow colonies from membranes in 44°C should be sub-cultured to Lauryl Tryptose Mannitol Broth CM831, incubated at 44°C to confirm gas production and indole production at this temperature after 24 hours incubation.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Escherichia coli ATCC® 25922
Negative control:
Bacillus subtilis ATCC® 6633

Precautions
Avoid overheating.

References

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MILK AGAR

Code: CM21

A nutrient medium enriched with milk solids for the determination of the viable micro-flora of dairy and water samples.

Formula	gm/litre
Yeast extract	3.0
Peptone	5.0
Milk solids (equivalent to 10ml fresh milk)	1.0
Agar	15.0
pH 7.2 ± 0.2	

Directions
Suspend 24g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Oxoid Milk Agar is made to a formula corresponding to the Official medium described in Dept. of Health Memo. 139/Foods[1]. It is recommended for performing the plate count test on milks, rinse waters, milk products and ice cream, etc.

It also complies with the recommendations of EUROGLACE (EEC Ice cream Industries) submitted to the EEC Commission for the examination of ice cream[2].

Statutory tests for milk must be carried out exactly as described in the appropriate Statutory Instrument e.g. Statutory Instrument 1963, Food & Drugs, Milk and Dairies: The Milk (Special Designation) (Amendment) Regulations. Ditto. (1965).

Technique
The sample bottle is inverted 25 times, and, after flaming the mouth, some of the milk sample is discarded – while the remainder is re-shaken thoroughly and used for the preparation of decimal dilutions in $\frac{1}{4}$ strength Ringer solution.

For milk, dilutions of 1/10, 1/100 and 1/1000 are prepared and 1ml of each pipetted aseptically into separate petri dishes. 10ml of molten Milk Agar, cooled to 45°C, is then added to each dish and the contents mixed by a combination of rapid to-and-fro shaking and circular movements lasting 5–10 seconds. The recommended procedure is five to-and-fro movements followed by five circular movements in a clockwise direction, succeeded by five to-and-fro movements at right angles to the first set followed by five anti-clockwise circular movements.

No more than fifteen minutes should elapse between preparation of the dilutions and pouring the plates.

The plates are allowed to stand on the bench for about an hour and then transferred to the incubator, where they are incubated in an inverted position for 2 days at 35°C or 3 days at 30°C.

Appreciably higher counts may be obtained after incubation at 22°C and 30°C than at 35°C[3,4,5].

After incubation the colonies are counted, within four hours, and the result expressed as plate count per ml.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Compare with previous lot/batch using pasteurised and raw milk samples, incubated at 32–35°C for 48 hours.

Precautions
Make sure that the procedures and media used in milk product testing comply with the National Regulations required for each country.

References

1 Dept. of Health (1987) *Memo. 139/Foods.*
2 Klose J. (1968) *Susswaren. 14. 778–782.*
3 Davis J. G. (1959) *'Milk Testing' 2nd ed., Dairy Industries Ltd., London, pp. 175–187.*
4 Thomas S. B. and Jenkins E. (1940) *Proc. Soc. Appl. Agric. 38–40.*
5 Wilson G. S. (1935) *'Bacteriological Grading of Milk' HMSO, London.*

MINERALS MODIFIED MEDIUM BASE

(SODIUM GLUTAMATE – L124)

Code: CM607

Formula (double strength)	gm/litre
Lactose	20.0
Sodium formate	0.5
L-cystine	0.04
L(–)aspartic acid	0.048
L(+)arginine	0.04
Thiamine	0.002
Nicotinic acid	0.002
Pantothenic acid	0.002
Magnesium sulphate $7H_2O$	0.200
Ferric ammonium citrate	0.020
Calcium chloride $2H_2O$	0.020
Dipotassium hydrogen phosphate	1.80
Bromocresol purple	0.020

pH 6.7 $\pm$ 0.1

Directions
Double Strength Dissolve 5 grams of Ammonium chloride in 1 litre of distilled water. To this add 22.7 grams of Minerals Modified Medium Base CM607, and 12.7 grams of Sodium glutamate L124. Mix to dissolve completely. Sterilise by autoclaving for 10 minutes at 116°C; alternatively heat to 100°C for 30 minutes on three successive days.

Single strength Dissolve 2.5 grams of Ammonium chloride in 1 litre of distilled water. To this add 11.4 grams of Minerals Modified Medium Base CM607, and 6.4 grams of Sodium glutamate L124. Mix to dissolve completely. Sterilise by autoclaving for 10 minutes at 116°C; alternatively heat to 100°C for 30 minutes on three successive days.

Note
To improve the stability of the dehydrated medium on storage the sodium glutamate L124 is supplied separately and must be added to the basal medium CM607.

The pH of the final medium is critical for optimum performance and the sterilised broth should be checked to confirm that it is at pH 6.7 before use.

Differences in heating procedures cause differences in final pH value. If necessary the heating procedure should be adjusted so that the final pH, after sterilisation is 6.7[1].

Description
A chemically defined medium based on glutamic acid was first advocated by Folpmers[2] for the enumeration of the coliform group of bacteria in water.

The Public Health Laboratory Service[3] carried out a trial and concluded that glutamic acid media containing glucose gave too many false positives in 48 hours. Gray[4] modified a glutamate medium containing lactose and later published a formulation for an improved Formate Lactose Glutamate Medium[5].

This latter medium was incorporated in another large trial carried out by the PHLS[6] in which three glutamate media were compared with Teepol Broth (Jameson & Emberly[7]) and MacConkey Broth. The results showed that Gray's improved formate lactose glutamate medium was superior to the other glutamate media on trial.

The report carried criticism of the mineral content of the medium and it was considered that it could be improved by modifying the amounts of minerals.

A co-operative investigation was carried out between the Metropolitan Water Board Laboratories and Oxoid Laboratories which resulted in a Minerals Modified Glutamate Medium CM289.

The Oxoid Minerals Modified Glutamate Medium was used in further PHLS[6] trials and the results with the Oxoid medium confirmed the superior performance of glutamate media reported previously (PHLS[6]).

The superior performance of Minerals Modified Glutamate Medium over MacConkey Broth is due mainly to improved detection of *Escherichia coli*. The table (adapated from PHLS[8]) illustrates the results obtained in the trial.

The table shows that for chlorinated water, incubation for >18 hours is required for glutamate media to demonstrate their superiority.

The medium and method are fully described in Her Majesty's Stationery Office Report 71[1].

More recently further trials showed Minerals Modified Glutamate Medium to be the medium of choice for the detection of *Esch. coli* in chlorinated waters, especially where the numbers of organisms concerned were small.

It was also found better than Lauryl Tryptose Lactose Broth for detection of small numbers of *Esch. coli* in other water, although the latter medium gave quicker results (18–24 hours compared to the 48 hours required by Minerals Modified Glutamate Medium).

Papadakis[10] investigated the isolation of *Esch. coli* from sea-water and found Minerals Modified Glutamate Medium to be better than MacConkey Broth formulations. However, to avoid high salt concentrations in the broth he recommended 1ml only of sea-water to be added to 10ml of single-strength MMG medium. Higher volumes of sea-water must be diluted out 1/10 with MMG medium.

Technique

The technique known as the Multiple Tube Method, Dilution Method or the Most Probable Number (MPN) method is used with Minerals Modified Glutamate Medium. A trial comparing membrane filtration and multiple tube methods showed glutamate medium to is unsatisfactory for use with membranes for enumerating coliform organisms in water[11].

With waters expected to be of good quality, the medium should be inoculated with one 50ml volume and five 10ml volumes. With waters of more doubtful quality, five 1ml volumes should be used in addition to the 50ml and 10ml volumes. Dilutions of the 1ml volumes may be required for polluted water and the 50ml volume may be omitted.

The larger volumes of water (10ml and 50ml) are added to equal volumes of double-strength medium, whereas the 1ml volumes (or dilutions of them) are added to 5ml of single-strength medium.

The tubes are incubated at 35°C and examined after 18–24 hours. All those tubes showing acid (yellow colour in the medium) and gas in the inverted inner (Durham) tube should be regarded as 'presumptive positive' tubes, including those in which gas appears after tapping the tube. The tube may only have a bubble of gas after tapping. The remaining tubes should be re-incubated and examined after another 24 hours. Any further tubes becoming 'positive' should be treated as 'presumptive positives'.

Each 'presumptive positive' tube should be sub-cultured to a tube of Brilliant Green Bile (2%) Broth CM31 and incubated for 24 hours at 44°C.

Comparison of Minerals Modified Glutamate Medium and MacConkey Broth by Numbers of Positive Tubes

	Number of tubes yielding								
	False positive reactions			Coliform organisms			*Esch. coli*		
	18hr	24hr	48hr	18hr	24hr	48hr	18hr	24hr	48hr
Unchlorinated samples									
MacConkey Broth	17	37	100	625	806	1060	467	528	582
Minerals Modified Glutamate Medium	2	20	97	557	858	1175	503	707	764
Chlorinated samples									
MacConkey Broth	4	19	49	125	216	315	77	121	128
Minerals Modified Glutamate Medium	0	1	37	59	223	395	39	144	203

At the same time a tube of 1% Tryptone Water CM87, should be inoculated for the production of indole after 24 hours at 44°C.

The production of gas from lactose at 44°C and the production of indole at 44°C are accepted in the United Kingdom as evidence of *Esch. coli*.

Samples of chlorinated water giving 'presumptive positive' tubes must be tested to exclude false positive results due to aerobic or anaerobic spore-bearing organisms that produce gas. Sub-cultures are made into Brilliant Green Bile (2%) Broth and incubated at 35°C for 48 hours. Production of gas within 48 hours can be taken as sufficient confirmation that coliform organisms are present. If the tubes are sub-cultured to MacConkey Agar CM7 at the same time, the colonial morphology of the organisms can easily be obtained for further differential tests.

A further multi-laboratory trial has demonstrated the efficiency of Lauryl Tryptose Mannitol Broth as a single tube confirmatory test of *Esch. coli*[12].

The Most Probable Number of organisms can be calculated from the tables in Appendix C of HMSO Report 71[1].

Modified Direct Plate Method for counting *Esch. coli* in food
A direct plate method (DPM) for the rapid enumeration of *Esch. coli*. in foods has been described[13]. This method was modified by a resuscitation procedure using Minerals Modified Glutamate Agar[14]. In the modified method 15g of agar per litre is added to Oxoid Minerals Modified Glutamate Broth CM607 plus L124. Using this resuscitation stage the authors have recovered damaged cells from frozen, dried, heat-processed or low pH foods.

Abbiss et al.[15] made a comparative assessment of the performance of Minerals Modified Glutamate Medium against three other enrichment broths in the enumeration of coliform organisms present in soft cheese, cooked meat and pâté. Minerals Modified Glutamate Medium was superior in sensitivity to Lauryl Sulphate Tryptose Broth, MacConkey Broth and Brilliant Green Bile Broth.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Escherichia coli (acid + gas) ATCC® 25922

Negative control:
Enterobacter aerogenes (acid only) ATCC® 13048

Precautions
Presumptive positive tubes must be sub-cultured to Lauryl Tryptose Mannitol Broth CM831 and incubated at 44°C to detect indole formation at this temperature before the identification of *Escherichia coli* can be made.

References

1 Departments of the Environment, Health & Social Security and PHLS (1982) *The Bacteriological Examination of Drinking Water Supplies. Report on Public Health and Medical Subjects No.71. HMSO, London.*
2 Folpmers T. (1948) *Ant. v. Leeuwenhoek, J. Microbiol. Serol. 14. 58–64.*
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5 Gray R. D. (1964) *J. Hyg. Camb. 62. 495–508.*
6 PHLS Standing Committee on Bacteriological Examination of Water Supplies (1968) *J. Hyg. Camb. 66. 67–82.*
7 Jameson J. E. and Emberly N. W. (1956) *J. Gen. Microbiol. 15. 198–204.*
8 PHLS Standing Committee on the Bacteriological Examination of Water Supplies (1969) *J. Hyg. Camb. 67. 367–374.*
9 Joint Committee of the PHLS and Standing Committee of Analysts (1980) *J. Hyg. Camb. 85. 35–48.*
10 Papadakis J.A. (1982) *6th Workshop on Marine Pollution of the Mediterranean, Cannes.*
11 PHLS Standing Committee on the Bacteriological Examination of Water Supplies (1972) *J. Hyg. Camb. 70. 691–705.*
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14 Holbrook R., Anderson J. M. and Baird-Parker A. C. (1980) *Food Technology in Australia, 32. 78–83.*
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MLCB AGAR

Code: CM783

Mannitol Lysine Crystal Violet Brilliant Green Agar for the isolation of salmonellae (not S. typhi or S. paratyphi A.).

Formula	gm/litre
Yeast extract	5.0
Peptone	10.0
'Lab-Lemco' powder	2.0
Sodium chloride	4.0
Mannitol	3.0
L-lysine hydrochloride	5.0
Sodium thiosulphate	4.0
Ferric ammonium citrate	1.0
Brilliant green	0.0125
Crystal violet	0.01
Agar	15.0
pH 6.8 ± 0.2	

Directions
Suspend 49.0 grams in litre of distilled water. Mix and bring gently to the boil with frequent agitation to dissolve the medium completely. Cool to 50°C and pour approximately 20ml into sterile petri dishes. DO NOT AUTOCLAVE OR OVERHEAT.

Description
Mannitol Lysine Crystal Violet Brilliant Green Agar (MLCB Agar) CM783 is based on the formula of Inoue et al.[1] for the selective isolation of salmonellae from faeces and foods. Visual detection of very small numbers of hydrogen sulphide-producing strains is easy because of the distinctive colonial appearance.

The concentration of Mg++ appears to be critical for maximum growth of salmonellae on MLCB Agar. van Schothorst et al.[2] showed that Oxoid MLCB Agar did not inhibit any of the Salmonellae species investigated.

Salmonellae serotypes that have a high incidence of H_2S-negative strains e.g. *S. sendai, S. berta, S. pullorum* and *S. senftenberg* may produce atypical pale colonies. MLCB Agar is not suitable for *S. typhi* and *S. paratyphi A*. because of the inhibitory concentration of brilliant green.

The medium may be inoculated directly with the specimen or from an enrichment culture. Selectivity is relatively weak and its performance may be adversely effected by heavily contaminated specimens. Because of these limitations MLCB Agar should not be used alone.

MLCB Agar is specified as a plating medium following enrichment in Modified Semi-Solid Rappaport Vassiliadis (MSRV) for isolation of *Salmonella* spp. from human faeces[3].

van Schothorst et al.[2] reported MLCB Agar to be excellent for the isolation of H_2S-positive salmonellae after enrichment in Rappaport-Vassiliadis (RV) Enrichment Broth CM669. They found that the selectivity of MLCB Agar was substantially increased after RV broth enrichment. They suggested Brilliant Green Agar and MLCB Agar should be used when examining heavily contaminated samples.

Salmonellae grow as large purple-black colonies due to hydrogen sulphide production. Mannitol is utilised by the organism and the resultant pH fall initiates lysine decarboxylation which controls further downward pH movement and promotes blackening.

MLCB Agar does not depend on lactose fermentation and is therefore recommended when investigating lactose-fermenting salmonellae (*Salm. arizona*).

Atypical Salmonella strains that produce little or no hydrogen sulphide grow as mauve-grey colonies and may develop a central black 'bulls eye'.

To assist the detection of these atypical strains Brilliant Green Agar (modified) CM329 or Bismuth Sulphite Agar CM201 should also be used.

Gram-positive and most Gram-negative organisms are inhibited although some strains of *Citrobacter* spp. may grow sufficiently well to mimic the appearance of *Salmonella* spp. and some *Proteus* spp. may swarm.

Most contaminating organisms that are able to grow develop as small colourless colonies.

Technique
Dry the surface of the agar before use.

Inoculate the medium heavily with the specimen or enrichment culture and incubate for 18–24 hours at 35°C.

Examine for typical large purple-black colonies of H_2S positive salmonella. Search carefully for H_2S negative strains that atypically grow as large mauve-grey colonies with a cratered centre. A proportion may show a black 'bulls eye'.

Pick all colonies presumed to be *Salmonella* spp. and confirm by biochemical and serological testing.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium at 2–8°C.

Quality Control
Positive control:
Salmonella virchow NCTC 5742

Negative control:
Escherichia coli ATCC® 25922

Precautions
The identity of colonies presumed from their appearance to be *Salmonella* spp. must be confirmed by biochemical and serological testing. In common with other enteric media care must be taken to ensure the purity of colonies taken for further testing as organisms that are inhibited from developing into colonies remain viable and may accidentally be picked on sub-culture.

Reference

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2 van Schothorst M., Renaud A. and van Beek C. (1987) *Food Microbiol. 4. 11–18.*
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MODIFIED LAURYL SULPHATE TRYPTOSE BROTH WITH MUG

Code: CM967

A modified Lauryl Tryptose Broth, incorporating 4-methylumbelliferyl-β-D-glucuronide (MUG) allowing the enumeration of presumptive Escherichia coli as well as other coliforms, using the Most Probable Number (MPN) method.

Formula	gm/litre
Tryptose	20.0
Lactose	5.0
Dipotassium hydrogen phosphate	2.75
Potassium dihydrogen phosphate	2.75
Sodium chloride	5.0
Sodium lauryl sulphate	0.1
4-methylumbelliferyl-β-D-glucuronide (MUG)	0.1
Tryptophan	1.0
pH 6.8 ± 0.2	

Directions
Dissolve 36.7g of Modified Lauryl Sulphate Tryptose Broth with MUG CM967 in 1 litre of distilled water.

Dispense into final containers containing Durham tubes.

Sterilise by autoclaving at 121°C for 15 minutes.

500g of this medium makes 13.3 litres of single strength medium.

Description
Oxoid Modified Lauryl Sulphate Tryptose Broth with MUG CM967 is formulated to allow use of the MPN technique for coliforms and also the enumeration of presumptive *E. coli* by means of a culture technique involving a liquid medium containing MUG.

The formulation contains 4-methylumbelliferyl-β-D-glucuronide (MUG), which is cleaved by the enzyme β-glucuronide to release 4-methylumbelliferone, a blue-green fluorophore exhibiting blue-green fluorescence visible when viewed under long wave ultra-violet (366nm). The inclusion of tryptophan acts as a substrate for indole production. Both reactions are characteristic of *E. coli* and can therefore be used to identify presumptive *E. coli.*

Coliform organisms will ferment lactose to produce gas. This production of gas can be taken as positive for the presence of coliforms.

Technique
Prepare a sufficient number of dilutions of original sample to ensure tubes for the final dilution will yield a negative result. Inoculate each dilution into tubes of Modified Lauryl Sulphate Tryptose Broth with MUG containing inverted Durham tubes. For the MPN technique, inoculate each dilution in triplicate.

Incubate the tubes at 30°C for 24–48 hours. Examine the tubes for growth turbidity, gas production, fluorescence and formation of indole. Read as follows:

1 Tubes showing fluorescence gas and formation of indole indicate presumptive *E. coli.*
2 Tubes showing gas formation indicate coliforms.

The MPN index can be determined from the numbers of positive tubes of selected dilutions by means of an MPB table, and a calculation of the MPN of presumptive *E. coli* or coliforms per gram or per millilitre of the original sample carried out.

Storage conditions and Shelf life
Modified Lauryl Sulphate Tryptose Broth with MUG CM967 should be stored tightly capped in the original container at 10°C–25°C. When stored as directed the medium will remain stable until the expiry date printed on the bottle.

Quality Control
Positive control:

Escherichia coli	ATCC® 25922

Negative control:

Staphylococcus aureus	ATCC® 25923
Enterobacter aerogenes	ATCC® 13048*

* also MUG-ve.

Precautions
Modified Lauryl Sulphate Tryptose Broth with MUG CM967 should only be used for *in vitro* diagnostic purposes.

Do not use beyond the stated expiry date, or if the product is caked, discoloured or shows any signs of deterioration.

References
1 IDF-170L (1994) Milie & Milie products. Enumeration of presumptive *Escherichia coli*.
2 ISO-11866–2: (1997) (E).

MODIFIED SEMI-SOLID RAPPAPORT VASSILIADIS (MSRV) MEDIUM BASE

Code: CM910

A semi-solid medium for the detection of motile Salmonella spp. from food and environmental samples.

Formula	gm/litre
Tryptose	4.59
Casein hydrolysate	4.59
Sodium chloride	7.34
Potassium dihydrogen phosphate	1.47
Magnesium chloride (anhydrous)	10.93
Malachite green oxalate	0.037
Agar	2.7
pH 5.2 ± 0.2	

MSRV SELECTIVE SUPPLEMENT

Code: SR161

Vial contents (each vial is sufficient to supplement 500ml of MSRV medium base)

Novobiocin	10mg

Directions
Suspend 15.8g of MSRV Medium Base in 500ml of distilled water. Bring to the boil with frequent agitation. DO NOT AUTOCLAVE. Cool to 50°C and aseptically add the contents of 1 vial of MSRV Selective Supplement reconstituted with 2ml of sterile distilled water. Mix well and pour into sterile petri dishes. Air dry at room temperature for at least one hour. (Plates may be air-dried overnight prior to storage at 2°C to 8°C.)

Description
Modified Semi-solid Rappaport Vassiliadis (MSRV) Medium is based on the formulation described by De Smedt et al which has been shown to detect more *Salmonella*-positive samples than the traditional enrichment procedures[1,2]. Further collaborative studies have confirmed these findings[3,4].

Motility enrichment on MSRV Medium has been designed as a simple, sensitive method for the isolation of salmonellae from food and environmental samples. The efficiency of the medium is based on the ability of salmonellae to migrate through the selective medium ahead of competing motile organisms, thus producing opaque halos of growth.

Further tests can be carried out directly from the migrated culture with the inoculum being taken from the edge of the growth. The Oxoid Salmonella Latex Test (FT203) is recommended for serological confirmation of *Salmonella* species.

The medium is not suitable for the detection of non-motile strains of Salmonella (incidence <0.1%)[5]. (Figures obtained from records of the Department of

Enteric Pathogens, Central Public Health Laboratory, Colindale, London. Dr. B. Rowe, Personal Communication, 1988.)

Technique

1 Inoculate three drops (ca. 0.1ml) of the pre-enrichment culture (after incubation for 16–20 hours) in separate spots on the surface of the MSRV Medium plates.

2 Incubate the plates in an upright position at 42°C for up to 24 hours. (Care should be taken not to exceed 24 hours.)

3 Examine the plates for motile bacteria which will be shown by a halo of growth originating from the inoculation spot.

4 Sub-cultures can be taken from the outside edge of the halo to confirm purity and for further biochemical and serological tests.

De Smedt[6] reported that if MSRV medium is contained in test tubes and incubation is carried out under anaerobic conditions, visible migration zones are produced in 6 hours enabling *Salmonella* in foods to be detected in 24 hours.

Storage conditions and Shelf life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the selective supplement in the dark at 2°C to 8°C and use before the expiry date on the label.

The prepared medium may be stored for up to 2 weeks at 2°C to 8°C in the dark.

Quality Control

Positive control:

Salmonella typhimurium ATCC® 14028 – Straw colonies at site of inoculation surrounded by halo of growth.

Salmonella enteritidis ATCC® 13076 – Straw colonies at site of inoculation surrounded by halo of growth.

Negative control:

Citrobacter freundii ATCC® 8090 – Restricted or no growth.

Precautions

The basal medium is very hygroscopic. When handling the powder a face mask and gloves must be worn.

References

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MRS AGAR

(DE MAN, ROGOSA, SHARPE)

Code: CM361

A solidified version of MRS Broth for the culture of 'lactic acid bacteria'.

Formula	gm/litre
Peptone	10.0
'Lab-Lemco' powder	8.0
Yeast extract	4.0
Glucose	20.0
Sorbitan mono-oleate	1ml
Dipotassium hydrogen phosphate	2.0
Sodium acetate $3H_2O$	5.0
Triammonium citrate	2.0
Magnesium sulphate $7H_2O$	0.2
Manganese sulphate $4H_2O$	0.05
Agar	10.0
pH 6.2 $\pm$ 0.2	

Directions

Suspend 62 grams in 1 litre of distilled water. Boil to dissolve the medium completely. Dispense into tubes, bottles or flasks and sterilise by autoclaving at 121°C for 15 minutes.

Description

The MRS formulation was developed by de Man, Rogosa and Sharpe[1] to replace a variable product (tomato juice) and at the same time to provide a medium which would support good growth of lactobacilli in general, even those strains which showed poor growth in existing media.

MRS medium is superior to the tomato juice medium of Briggs[2] and the meat extract tomato juice medium of de Man. It gives more profuse growth of all strains of lactobacilli, especially the difficult and slow growing strains of *L. brevis* and *L. fermenti*.

MRS Agar and Broth were designed to encourage the growth of the 'lactic acid bacteria' which includes species of the following genera: *Lactobacillus, Streptococcus, Pediococcus* and *Leuconostoc*. All these species can produce lactic acid in considerable amounts. They are Gram +ve, catalase and oxidase –ve and are fastidious in their nutritional requirements. Growth is enhanced considerably by micro-aerophilic conditions.

Generally the 'lactic acid bacteria' show delayed growth and smaller colony size than other micro-organisms. They may be overgrown in non-selective media, especially if incubation is required for 2–4 days.

Selection can be made by pH adjustment, thus lactobacilli will tolerate lower pH levels than streptococci (pH 5.0–6.5) with pediococci and leuconostocs growing best within this range. Inhibitors of the main groups of competitor microflora include thallous acetate, sodium acetate, sorbic acid, acetic acid, sodium nitrite, cycloheximide and polymyxin. These substances can be used at varying concentrations and combinations but inevitably a compromise has to be reached between selectivity and productivity of the organism sought[3].

MRS Agar with sorbic acid has been described[3,4]. This is MRS medium with its pH reduced to 5.7 and 0.14% w/v sorbic acid added (=0.2% w/v potassium sorbate).

An evaluation of media for selective enumeration of *Lactobacillus acidophilus* and *Bifidobacterium* species showed that minor adjustments to the basic formula of MRS Agar can readily be made to optimise its performance for determining the content of *L. acidophilus* and *Bifidobacterium* spp. in the presence of other lactic acid bacteria which are present in yoghurt[5].

The lactobacilli are micro-aerophilic and generally require layer plates for aerobic cultivation on solid media. Submerged or surface colonies may be compact or feathery, and are small, opaque and white.

Technique
Products to be examined for lactobacilli content are macerated or diluted in a diluent such as quarter-strength Ringer solution, and further dilutions are made in MRS Broth.

1ml volumes of the diluted samples are added to sterile petri dishes, and molten MRS Agar (45°C) is poured into the dish and mixed thoroughly.

When the medium has set, another layer of uninoculated MRS Agar is poured over the surface to produce a layer-plate.

Plates are incubated as described below. It is important that adequate moisture vapour is present in the atmosphere above the agar because drying of the plates during incubation will concentrate the selective factors on the surface and make the medium inhibitory. The presence of carbon dioxide stimulates growth and plates should be incubated in an atmosphere of 5% CO_2. MRS medium is selective for lactobacilli but some growth of leuconostocs and pediococci may occur.

Incubation method
42°C thermophilic: 2 days
35°C mesophilic: 2 days
30°C + 22°C mesophilic-psychrotrophic: 2+1 days
25°C psychrotrophic: 3 days

Incubation carried out under anaerobic or micro-aerophilic conditions
Select isolated colonies on the agar medium and stain a smear from each to identify the presumptive *Lactobacillus* colonies; pick these off into MRS Broth. An advantage of this broth is that any other micro-organisms, originally lying dormant in the selective agar, are not given the opportunity to multiply, as may occur in a non-selective broth. Incubate the broths at temperatures and times similar to those used for the MRS Agar; they can then be examined microscopically and further sub-cultured to MRS Agar for subsequent confirmation and identification of species.

References

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3 Reuter G. (1985) *Intern. J. Food Microbiol. 2. 55–68.*
4 ISO/TC 34/SC 6/WG 15, No.3 and 5 (1984) *Enumeration of Lactobacteriaceae in meat and meat products.*
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MRS BROTH (DE MAN, ROGOSA, SHARPE)

Code: CM359

A non-selective medium for profuse growth of 'lactic acid bacteria'.

Formula	gm/litre
Peptone	10.0
'Lab-Lemco' powder	8.0
Yeast extract	4.0
Glucose	20.0
Sorbitan mono-oleate	1ml
Dipotassium hydrogen phosphate	2.0
Sodium acetate $3H_2O$	5.0
Triammonium citrate	2.0
Magnesium sulphate $7H_2O$	0.2
Manganese sulphate $4H_2O$	0.05
pH 6.2 $\pm$ 0.2	

Directions
Add 52 grams to 1 litre of distilled water at 60°C. Mix until completely dissolved. Dispense into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
MRS Broth may be used for tests in the identification of lactobacilli, such as temperature dependence, growth in 4% NaCl, growth in 0.4% Teepol, etc. as recommended by Sharpe, Fryer and Smith[1].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Lactobacillus gasseri ATCC® 19992

Negative control:
Staphylococcus aureus ATCC® 25923

Reference

1 Sharpe M. Elisabeth, Fryer T. F. and Smith D. G. (1966) *'Identification of the Lactic Acid Bacteria' in 'Identification Method for Microbiologists Part A' (Gibbs B. M. and Skinner F. A. eds.) London and New York, Academic Press. Pages 65–79.*

MRVP MEDIUM

Code: CM43

A medium recommended for the Methyl-red and Voges-Proskauer tests for the differentiation of the coli-aerogenes group.

Formula	gm/litre
Peptone	5.0
Glucose	5.0
Phosphate buffer	5.0
pH 7.5 ± 0.2	

Directions
Add 15g to 1 litre of distilled water. Mix well, distribute into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
This glucose-phosphate medium is recommended for the Methyl-red and Voges-Proskauer tests, for the differentiation of the coli-aerogenes group[1].

Smith[2] noted the low acid production of *Enterobacter aerogenes* cultures as compared with those of *Escherichia coli*. Clark & Lubs[3] employed methyl-red as a hydrogen-ion concentration indicator in order to differentiate glucose phosphate peptone water cultures of members of the coli-typhoid group. This test, now known as the Methyl-red test, distinguishes those organisms able to form large amounts of acid from glucose so that the pH falls below 4.4 and those organisms which cannot produce a low pH level.

The difference in pH value is visualised by adding methyl-red to the culture, (<pH 4.4 red: pH 5.0–5.8 orange: >pH 6.0 yellow).

Methyl-red reaction

Colour	**Organism**
Orange to red (MR positive)	*Escherichia coli* *Citrobacter* species and others.
Orange to yellow (MR negative)	*Enterobacter* species *Klebsiella pneumoniae* and others.

Voges & Proskauer[4] described a red fluorescent coloration which appeared after the addition of potassium hydroxide to cultures of certain organisms in glucose medium. The coloration was shown to be due to the oxidation of the acetylmethyl-carbinol producing diacetyl which reacts with the peptone of the medium to give a red colour[5,6]. Durham[7] noted that *Ent. aerogenes* gave a positive reaction but that *Escherichia coli* produce no coloration, and it later became clear that there was a negative correlation between the Methyl-red and Voges-Proskauer tests[8,9] for lactose-fermenting coliform organisms.

Voges-Proskauer reaction

Red (Positive)	*Enterobacter* species and others
No colour (Negative)	*Escherichia coli* and others.

Technique
Inoculate a 10ml tube of MRVP Medium with two loopfuls of a pure, 4–6 hours old, Peptone Water CM9 culture of the organism under test.

Incubate not less than 48 hours at 35°C for the MR test but more usually 3–5 days at 30°C. A heavy inoculum and 18–24 hours incubation at 35°C may give a rapid result[10]. A rapid VP test may be carried out from a heavy inoculum and incubation in a water bath at 35°C for 4–5 hours. Some organisms (*Hafnia alvei*) require incubation at 25°C to give a positive VP test.

After incubation, test one portion of the broth with 5 drops of 0.4% w/v methyl-red solution and read the colour on the surface of the medium immediately.

The second portion of the broth is used for the VP reaction by one of the following methods:

1 Add 3ml of 5% w/v alcoholic α-naphthol solution and 3 ml of 40% w/v KOH solution (Barritt's method[12]).
2 Add a trace amount of creatine (2 drops of a 0.3% w/v solution) and 5ml of 40% KOH solution (O'Meara's method 13).

A bright pink or eosin red colour will appear after gentle shaking for 30 seconds. A pink colour is positive; no colour is negative.

Barry & Feeney[14] obtained rapid results by adding creatine to Barritt's reagents.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–6°C.

Quality Control
Positive MR:
Escherichia coli ATCC® 25922

Positive VP:
Enterobacter cloacae ATCC® 23355

Negative MR:
Klebsiella pneumoniae ATCC® 13883

Negative VP:
Escherichia coli ATCC® 25922

Precautions
The MR-VP reactions are only part of the tests required to identify organisms.

Each laboratory should standardise on the inoculum density, volume of broth and the test container size.

MR tests require a minimum incubation of 48 hours before the pH indicator is added.

When using Barritt's reagents add α-naphthol first and KOH second; do not reverse this order.

Vaughn et al.[15] warned of false positive VP reactions if the completed tests are left standing for over an hour.

References

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MUELLER-HINTON AGAR

Code: CM337

An antimicrobial susceptibility testing medium which may be used in internationally recognised standard procedures.

Formula*	gm/litre
Beef, dehydrated infusion from	300.0
Casein hydrolysate	17.5
Starch	1.5
Agar	17.0
pH 7.4 ± 0.2	

* modified to meet performance standards.

Directions
Add 38 grams to 1 litre of distilled water. Bring to the boil to dissolve the medium completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Mueller-Hinton Agar was designed to be a reproducible culture medium for the isolation of pathogenic *Neisseria* species (Mueller & Hinton[1]). The inclusion of starch ensures that toxic factors found during growth will be absorbed and its presence is often essential to establish growth from very small inocula[2].

However, GC selective media (GC Agar Base CM367 plus supplements SR56/SR91/SR95/SR101/SR104/SR105) have replaced Meuller-Hinton Agar for this purpose.

The major use of Mueller-Hinton Agar is for Antimicrobial Susceptibility Testing (AST). It has become the standard medium for the Bauer-Kirby method[3,4] and its performance is specified by the NCCLS[5,6].

Oxoid Mueller-Hinton Agar meets the requirements of WHO[7,8]. Criticisms have been made about variation in performance of Mueller-Hinton Agar between and with manufacturers' batches/lots of medium[9]. The causes of such variation are:

1 The effects of differences in concentration of divalent cations Mg++ and Ca++. These effects are shown as MIC variations with aminoglycosides against *Pseudomonas aeruginosa* and tetracycline against staphylococci[10,11,12].
2 Variation in thymine and thymidine content, which affect sulphonamide and trimethoprim MIC values[13,14].
3 Differences in the characteristics of the agar used in the medium, especially diffusion properties[15].

In the light of such criticisms the NCCLS called interested manufacturers together to discuss the standardisation and stabilisation of Mueller-Hinton Agar. Control methods were established whereby critical antimicrobial/organism combinations had to yield consistent zones of inhibition within 2mm of the specified diameters in the standards[6].

The result of this cooperative effort is that Mueller-Hinton Agar is now a standard medium and declares on the label that it conforms to the NCCLS standard M6-A.

'This lot meets the NCCLS standard M6-A for dehydrated Mueller-Hinton agar.'

For further details of antimicrobial susceptibility testing see Section 6.

Mueller-Hinton Agar supplemented with yeast, NAD and haematin is used specifically for the susceptibility testing of *Haemophilus influenzae*[16]. For further details see Haemophilus Test Medium (HTM), CM989.

Mueller-Hinton Agar and Broth are used as the basis of solid and liquid media containing cefoperazone, trimethoprim, piperacillin and cycloheximide for selective isolation of *Arcobacter* spp. from meats[16].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control
Positive controls:
Escherichia coli ATCC® 25922
Pseudomonas aeruginosa ATCC® 27853
Staphylococcus aureus ATCC® 25923
Enterococcus faecalis ATCC® 29212

Negative control:
Uninoculated medium.

Precautions
Incubation in a CO_2 enriched atmosphere is not recommended because of its pH effect on the medium. If it is imperative to use CO_2 then known control organisms should be included with the test plates to measure its effect.

Carbohydrates should not be added to Mueller-Hinton Agar because they may influence the rate of growth of the organism and the resulting pH of the medium.

The addition of lysed horse blood to the medium may further reduce the levels of thymidine and prevent the growth of thymidine-dependent organisms.

References
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MUELLER-HINTON BROTH

Code: CM405

An antimicrobial susceptibility testing medium which may be used in internationally recognised standard procedures.

Formula*	gm/litre
Beef, dehydrated infusion from	300.0
Casein hydrolysate	17.5
Starch	1.5
pH 7.3 ± 0.1	

* modified to meet performance standards.

Directions
Dissolve 21g in 1 litre of distilled water. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Oxoid Mueller-Hinton Broth has been produced in parallel with Oxoid Mueller-Hinton Agar CM337. Where studies on antibiotic susceptibilities are being made both in broth and agar, it will be found to be of particular value to have media of identical nutrient formulation.

Mueller-Hinton Broth is recommended for broth dilution MIC studies[1].

Oxoid Mueller-Hinton Broth will require supplementation with the divalent cations Mg++ and Ca++ after sterilisation[2].

Lysed horse blood or thymidine phosphorylase may be added to the broth to improve the MIC endpoints of sulphonamides and trimethoprim[3].

Mueller-Hinton Broth containing horse serum and agar added to create a semi-solid agar medium was used in microtitre plates in an agar dilution method for determining the MIC for *Helicobacter pylori* of a number of antibiotics. The method does not require prolonged incubation in carbon dioxide-enriched air and results are available in 48 hours compared to 3–4 days for agar diffusion testing on solid medium[4].

For further details of antimicrobial susceptibility testing see Section 6.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Escherichia coli ATCC® 25922
Pseudomonas aeruginosa ATCC® 27853
Staphylococcus aureus ATCC® 25923
Enterococcus faecalis ATCC® 29212

Negative control:
Uninoculated medium

Precautions
Monitor the performance of the broth routinely using the standard QC organisms. If the broth does not yield the expected MIC values, modify the volumes of Mg++ and Ca++ solutions until the MIC values approximate to those in Table 3 in reference[1].

If the thymidine content is lowered, after the addition of lysed horse blood or thymidine phosphorylase, the MIC values may be lower.

References
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MULLER-KAUFFMANN TETRATHIONATE BROTH BASE

Code: CM343

An improved enrichment medium for the isolation of salmonellae and the suppression of Proteus species.

Formula	gm/litre
Tryptone	7.0
Soya peptone	2.3
Sodium chloride	2.3
Calcium carbonate	25.0
Sodium thiosulphate	40.7
Ox bile	4.75

Directions
Suspend 82 grams in 1 litre of distilled water and bring to the boil. Cool below 45°C and add, just prior to use, 19ml of iodine solution and 9.5ml of a 0.1%

brilliant green solution. Mix well and fill out into sterile tubes or flasks.

Iodine Solution

Iodine	20 grams
Potassium iodide	25 grams
Distilled water to	100ml

Dissolve the potassium iodide in approximately 5ml of distilled water, add the iodide and gently warm the solution to completely dissolve it. Make up the volume to 100ml with distilled water.

Brilliant Green Solution

Brilliant Green (BDH or Chroma)	0.1 grams
Distilled water	100ml

Add the brilliant green to the distilled water and shake to dissolve the dye. Heat the solution to 100°C for 30 minutes and shake from time to time whilst cooling, to ensure that the dye has completely dissolved. Store in a brown glass bottle or away from light.

Description
Muller[1] developed this medium in 1923. It was later modified by Kauffmann[2,3] with the addition of brilliant green and ox bile to suppress commensal organisms and thus improve the isolation of salmonellae.

The brilliant green dye used in the medium has been shown to be critical and Chroma or BDH brands should be used. It is essential that the dye is added as directed because heating the brilliant green or attempting to incorporate it in the basal medium seriously impairs its selective action.

The addition of novobiocin at 40mg per litre of broth was described by Jeffries[4] to suppress the growth of *Proteus* species.

Muller-Kauffmann Tetrathionate Broth should not be used if *Salmonella typhi* is suspected.

Muller-Kauffmann Tetrathionate Broth was used in a large-scale investigation between nine laboratories in eight different countries[5].

Incubation of Muller-Kauffmann Broth at 43°C was shown to be essential in this trial and the technique used for enrichment of the salmonellae is as follows:

Add approximately 10 grams of sample to 100ml of Muller-Kauffmann Broth. Shake vigorously and immediately place the flasks of medium in a 45°C water-bath for 15 minutes. Remove the flasks from the water-bath, without drying them, and place in an incubator or another water-bath at 43°C.

Sub-culture the broth after 18–24 hours and again after 48 hours. Take one loopful of broth from the edge of the surface of the fluid and inoculate either two Oxoid Brilliant Green Agar (Modified) CM329 plates (9cm diameter) without recharging the loop between plates, or one large plate (14cm diameter).

Incubate the plates at 35°C for 18–24 hours.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922

Precautions
Do not autoclave the base broth.

Add the iodine solution and brilliant green just prior to use.

The medium is not suitable for the growth of *S. typhi*, *S. sendai*, *S. pullorum* and *S. gallinarum*.

References

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MYCOPLASMA AGAR BASE

Code: CM401

A basic medium which, after enrichment with supplements, will support the growth of Mycoplasma species.

Formula	gm/litre
Bacteriological peptone	10.0
'Lab-Lemco' powder	10.0
Sodium chloride	5.0
Mineral supplement	0.5
Agar	10.0
pH 7.8 ± 0.2	

Directions
Add 35.5g to 1 litre of distilled water. Boil to dissolve the agar and distribute in 80ml volumes. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C, and add the sterile supplement.

MYCOPLASMA SUPPLEMENT-G

Code: SR59

POISON – CONTAINS THALLIUM SALT

Vial contents (each vial is sufficient for 80ml of medium)

Horse serum	20ml
Yeast extract (25% w/v)	10ml
Thallous acetate	25mg
Penicillin	20,000 IU

Directions
The sterile supplement is prepared by aseptically adding 20ml of sterile distilled water to the vial and mixing gently. Add this to 80ml of sterilised Oxoid Mycoplasma Agar or Broth Base CM401/CM403, previously cooled to 50°C.

MYCOPLASMA SUPPLEMENT-P

Code: SR60

POISON – CONTAINS THALLIUM SALT

Vial contents (each vial is sufficient for 80ml of medium)

Horse serum	6ml
Yeast extract (25% w/v)	3ml
Thallous acetate	0.008g
Glucose	0.3g
Phenol red	0.0012g
Methylene blue chloride	0.0003g
Penicillin	12,000IU
Mycoplasma Broth Base CM403	0.145g

Directions
Prepare the sterile supplement by aseptically adding 20ml sterile distilled water to the vial and mix gently. Add 1ml of Oxoid Mycoplasma Base CM401 without supplements to each of ten small bottles. Sterilise by autoclaving at 121°C for 15 minutes. Allow to set. Aseptically add 2ml of the reconstituted Supplement-P to each bottle containing agar.

Description
Oxoid Mycoplasma Agar Base CM401 was formulated as a basal medium to be enriched with any satisfactory supplementary factors used for the growth of mycoplasmas (PPLO).

Edward[1] stressed the importance of the absence of toxic factors to mycoplasmas in the basal medium. Lynn & Morton[2] paid special attention to the inhibitory factors which can be present in batches of agar. Oxoid Mycoplasma Agar Base contains selected constituents shown to be free from such inhibitory or toxic substances. It also contains a special mineral supplement which improves the growth and colony characteristics of mycoplasmas without interfering with the clarity of the medium.

The basal medium should be supplemented with 10% v/v of a 25% w/v extract of baker's yeast (Hayflick[3]) or Oxoid Yeast Extract L21 (Lemcke[4]). The majority of mycoplasmas require media enriched with serum; horse serum (20% v/v) is commonly used. Swine or human sera may be substituted for horse serum but the possible presence of antibodies or antibiotics in human serum make media control of great importance (Fallon[6]). The addition of DNA to the medium to encourage the growth of bovine general genital strains and other mycoplasmas was suggested by Edward[7]. 20μg of sodium deoxyribonucleate per ml of medium is quoted by Lemcke[4].

Antibacterial agents are necessary to prevent overgrowth of the slow-growing mycoplasmas by contaminating organisms. Penicillin and thallous acetate are the most common agents used but T-strain mycoplasma* are sensitive to thallous acetate. Hutchinson[5] and Fallon[6] state that ampicillin at 1mg per ml of medium may be substituted for penicillin and thallous acetate.

Penicillin may be used at concentrations between 50 and 500 units per ml and thallous acetate between 1/2000 and 1/8000 (Lemcke)[4]. It is preferable to omit thallous acetate when searching for T-strain mycoplasma* (Shepherd & Lanceford[8]).

The two pre-sterilised supplements, Mycoplasma Supplement-G and Mycoplasma Supplement-P have been developed for the improved growth of mycoplasmas. Mycoplasma Supplement-G is a general supplement prepared to the formulation of Hayflick[3] which, when added to Oxoid Mycoplasma Broth or Agar Base produces a complete selective medium for the propagation of sterol-requiring *Mycoplasma* species of the classical type.

Mycoplasma Supplement-P is a liquid supplement based on the formulation recommended by the Mycoplasma Reference Laboratory, CPHLS, Colindale, which is used in conjunction with Mycoplasma Agar Base to form a bi-phasic medium for the isolation and preliminary identification of *Mycoplasma pneumoniae*.

Many species of mycoplasmas are aerobes or facultative anaerobes but some prefer micro-aerophilic conditions with the addition of carbon dioxide, or strict anaerobiosis.

Pathogenic strains grow best at 35°C while saprophytic strains often grow between 22°C and 30°C, T-strains* have an optimal temperature of 36°C.

Mycoplasma species grow best at pH 7.4–8.0 but T-strains* prefer pH 6.0–6.5.

**** T-strain mycoplasma = Ureaplasma urealyticum***

Material for cultivation is inoculated into broth or agar medium overlaid with broth. Agar plates are incubated in moist chambers aerobically, anaerobically and in 10% CO_2–90% N_2 atmosphere. Examine the agar surface after 7 days incubation with a dissecting microscope at 60x magnification, using obliquely transmitted light. The colonies are characteristic with the centre of the colony embedded beneath the surface, giving a 'fried-egg' appearance.

Purification of the organism by further cloning sub-cultures is essential before identification. This may be carried out by removing a plug of agar containing a colony from the plate and using it to inoculate further plates of medium.

Growth inhibition tests using specific antisera may then be carried out (Clyde[9]).

Bi-phasic medium bottles should be inoculated with a swab or a fleck of sputum and incubated at 35°C for up to three months. Any bottles showing gross turbidity due to growth of bacteria or fungi should be discarded.

Growth of *M. pneumoniae* results in the reduction of methylene blue followed by production of acid due to the fermentation of glucose, resulting in a colour change of the phenol red indicator to yellow. Bottles showing such a colour change should be sub-cultured onto agar for further examination. Oxoid Mycoplasma Agar Base supplemented with Oxoid Mycoplasma Supplement-G SR59 is suitable for this purpose.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Mycoplasma pneumoniae ATCC® 15531

Negative control:
Ureaplasma urealyticum (when thallous acetate is present in the medium) ATCC® 27618

Precautions
Thallous acetate is toxic, observe the precautions stated under HAZARDS page 2–7.

Do not use thallous acetate media to isolate *U. urealyticum*.

Mycoplasmas may be suspected if (1) typical morphology (2) no growth in media without serum (3) colonies are embedded below the agar surface.

References

1 Edward D. G. ff. (1971) *J. Gen. Microbiol. 69. 205–210.*
2 Lynn R. J. and Morton H. E. (1965) *Appl. Microbiol. 4. 339–341.*
3 Hayflick L. (1965) *Texas Rep. Biol. & Med. 23. suppl. 1. 285–303.*
4 Lemcke Ruth M. (1965) *'Media for the Mycoplasmataceae', Lab. Pract. 14. 712.*
5 Hutchinson D. (1969) *J. Med. Lab. Technol. 26. 111–116.*
6 Fallon R. J. (1969) *S. A. B. Technical series 3. Academic Press. 41–50.*
7 Edward D. G. ff. (1954) *J. Gen. Microbiol. 10. 27–64.*
8 Shepard M. C. and Lanceford C. D. (1970) *Appl. Microbiol. 2. 539–543.*
9 Clyde W. A. (1964) *J. Immun. 92. 958–962.*

NEW YORK CITY MEDIUM

NYC Medium, primarily designed for the isolation of pathogenic Neisseria, also readily supports the growth of Mycoplasma and Ureaplasma species.

The transparent, highly selective medium permits direct microscopic observation and presumptive recognition of mycoplasmas. As a single medium it can, therefore, be doubly useful in the diagnosis of gonorrhoea and in the recognition of active or asymptomatic mycoplasma infections.

Urine specimens can be cultivated on NYC Medium to detect the presence of *Ureaplasma* species (T-strain mycoplasma).

References

1 Faur Y. C., Weisburd M. H., Wilson M. E. and May P. S. (1974) *Appl. Microbiol. 27. 1041–1045.*
2 Hipp S. S., Rockwood L. D., Gaafar H. A. and Han Y. (1981) *J. Clin. Microbiol. 13. 135–138.*

MYCOPLASMA BROTH BASE

Code: CM403

A basic medium which, after enrichment with supplements can be used in the isolation and cultivation of mycoplasmas from clinical specimens.

Formula	gm/litre
Bacteriological peptone	10.0
'Lab-Lemco' powder	10.0
Sodium chloride	5.0
Mineral supplement	0.5
pH 7.8 ± 0.2	

Directions
Dissolve 25.5 grams in 1 litre of distilled water. Mix well and distribute in 80ml volumes. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add the sterile supplement.

Description
Oxoid Mycoplasma Broth Base CM403 complements Oxoid Mycoplasma Agar Base CM401.

It requires supplementation with yeast extract, serum and antibiotics, as described for Oxoid Mycoplasma Agar Base.

Carbohydrate fermentation by mycoplasmas can be tested by incorporating 1% w/v carbohydrate and 0.005% w/v phenol red into the broth medium.

A *Ureaplasma* broth[1] can be prepared by adding to 95ml broth medium (pH adjusted to 6.0):

Horse Serum	4.ml
Urea	0.05g
Phenol red	0.001g
Penicillin	100,000 units

A similar medium was described by Taylor-Robinson et al.[2] where reference is made to the use of HEPES buffer to induce large colony-forming *Ureaplasma* strains and for the isolation and titration of viable mycoplasma by the metabolism of arginine or glucose and measuring the pH change in the medium.

A selective medium for *Mycoplasma pneumoniae* was described by Kraybill & Crawford[3].

Mycoplasma Supplement-G SR59 and Mycoplasma Supplement-P SR60 may be used with this medium – see Mycoplasma Agar Base CM401.

Most strains of mycoplasmas are encouraged by growth in a biphasic medium in which a layer of Broth medium covers a basal layer of Mycoplasma Agar. (Reference Mycoplasma Agar Base CM401.)

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Mycoplasma pneumoniae ATCC® 15531
Ureaplasma urealyticum (if thallous acetate is absent)

Negative control:
Escherichia coli ATCC® 25922

Precautions
Thallous acetate is toxic, observe the precautions stated under Hazards on page 2–7.

Sub-culture the broth to agar as soon as the indicator begins to change colour before the pH change destroys the organism.

References

1 Shepard M. C. and Lanceford C. D. (1970) *Appl. Microbiol. 20. 539–543.*
2 Taylor-Robinson D., Martin-Bourgon C., Watanable T. and Addey J. P. (1971) *J. Gen. Microbiol. 68. 97–107.*
3 Kraybill W. H. and Crawford Y. E. (1965) *Proc. Soc. Exp. Biol. Med. 118. 965–967.*

NUTRIENT AGAR

Code: CM3

A general purpose medium which may be enriched with 10% blood or other biological fluid.

Formula	gm/litre
'Lab-Lemco' powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
Agar	15.0
pH 7.4 ± 0.2	

Directions
Suspend 28g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Nutrient Agar is a basic culture medium used to sub-culture organisms for maintenance purposes or to check the purity of sub-cultures from isolation plates prior to biochemical or serological tests.

In semi-solid form, agar slopes or agar butts the medium is used to maintain control organisms[1].

Nutrient Agar is suitable for teaching and demonstration purposes. It contains a concentration of 1.5% of agar to permit the addition of up to 10% of blood or other biological fluid, as required. The medium, without additions, may be used for the cultivation of organisms which are not exacting in their food requirements.

For a medium which is richer in nutrients, see Blood Agar Base No.2 CM271.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923
Escherichia coli ATCC® 25922

Negative control:
Uninoculated medium

Reference

1 Lapage S. P., Shelton J. E. and Mitchell T. G. (1970) *in 'Methods in Microbiology' Eds. Norris J. R. and Ribbons D. W. Vol.3A. Academic Press. London. p.116.*

NUTRIENT BROTH

Code: CM1

A general purpose fluid medium for the cultivation of micro-organisms not exacting in their nutritional requirements. Blood, serum, sugars, etc., may be added as required for special purposes.

Formula	gm/litre
'Lab-Lemco' powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
pH 7.4 ± 0.2	

Directions
Add 13g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Lab-Lemco beef extract is combined with peptone and sodium chloride to form the basic bouillon described by Loeffler and other early bacteriologists. Yeast extract is added to provide vitamins and minerals to help speed the growth of most organisms.

Nutrient Broth can be enriched with other ingredients such as carbohydrates, blood etc., for special purposes. See also Nutrient Broth No.2 CM67.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium below 25°C.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923
Escherichia coli ATCC® 25922

Negative control:
Uninoculated medium

NUTRIENT BROTH NO.2

Code: CM67

A nutritious medium suitable for the cultivation of fastidious pathogens and other micro-organisms.

Formula	gm/litre
'Lab-Lemco' powder	10.0
Peptone	10.0
Sodium chloride	5.0
pH 7.5 ± 0.2	

Directions
Add 25g to 1 litre of distilled water. Mix well, distribute into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
This medium is comparable to a meat infusion and is richer in nutrients than Nutrient Broth CM1. It gives good growth from small inocula and is recommended for sterility testing for aerobic organisms. Nutrient Broth No.2 complies with the recommendations in the 'British Pharmacopoeia'[1] for the composition of a sterility testing medium for aerobes. The medium is ideally suited for sub-culture, particularly as a secondary growth medium for staphylococci which are to be tested for coagulase production. Nutrient Broth No.2 made up at double strength corresponds to the medium recommended by the British Standards Institution[2] for use in the determination of the Rideal-Walker Coefficient of Disinfectants.

Nutrient Broth No.2 is the basal medium for Preston Campylobacter Enrichment Broth[3,4].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923
Pseudomonas aeruginosa ATCC® 27853

Negative control:
Uninoculated medium

References

1 *British Pharmacopoeia* (1980) London HMSO.
2 British Standard 541: (1934) *'Determining the Rideal Walker Coefficient of Disinfectants' BSI London, p.9.*
3 Bolton F. J. and Robertson L. (1982) *J. Clin. Pathol. 35. 462–467.*
4 Bolton F. J., Coates D. and Hutchinson D. N. (1984) *J. Appl. Bact. 56. 151–157.*

NUTRIENT GELATIN (CM135a)

Code: CM635

For determination of gelatin liquefaction, and for the 20°C plate count.

Formula	gm/litre
'Lab-Lemco' powder	3.0
Peptone	5.0
Gelatin	120.0
pH 6.8 ± 0.2	

Directions
Suspend 128g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring and cool below 20°C or leave to set in a refrigerator.

Description
Nutrient Gelatin CM635, a solid medium at 22°C and below, is employed for the determination of gelatin liquefaction and for the 20°C plate count[1].

Gelatin is liquefied in a characteristic manner by certain proteolytic organisms; whereas gelatin media have been largely superseded by agar media for most purposes, nutrient gelatin is still employed for differentiation of micro-organisms by their proteolytic effects.

Technique
Test for gelatin liquefaction by incubating a Nutrient Gelatin stab or plate culture at 20–22°C. Alternatively, incubate at a higher temperature (usually optimum for the organism under investigation) and then transfer the tube to a refrigerator or into cold water before observation. The latter method not only allows determinations to be carried out on organisms which grow slowly or not at all at 20–22°C but also usually avoids false positive results produced by the release of enzymes after the death of the organisms[2].

If the medium is incubated at a higher temperature it is necessary to employ uninoculated controls to allow for the hydrolytic effect of heat and other factors. Rates of liquefaction vary considerably, so that some organisms produce liquefaction within a few days whereas others require several weeks. For practical purposes, a maximum incubation period of 14 days is suggested[3,4].

Considerably longer incubation may be necessary, some strains of *Enterobacter cloacae* liquefied gelatin only after 3 months at 20–22°C[5]. Especially where prolonged incubation is necessary, it is important to ensure adequate closure of the containers in order to prevent dehydration of the medium. Besides its presence or absence, the shape and nature of the liquefied portion of the stab culture are often useful identifying characteristics.

Particularly with plate cultures, gelatin liquefaction may be detected sooner by the 'Stone reaction' (Stone[6]): add a drop of saturated aqueous ammonium sulphate solution, or of fresh 20% aqueous sulphosalicylic acid solution, to an individual colony growing on Nutrient Gelatin. A positive reaction (i.e. gelatin liquefaction) is indicated by the presence of a clear zone round the colony after 10 minutes contact with either reagent. This method is slightly less sensitive but several strains may be tested on one plate. The 'Stone reaction' is also employed with Staphylococcus Medium No.110, CM145 for the differentiation of staphylococci.

For the standard gelatin plate count on water (American Public Health Association[1]) dilute the original sample with sterile tap water and place 0.5 or 1ml of the dilutions in each dish of at least two duplicate sets of sterile petri dishes. Cool the sterile prepared Nutrient Gelatin to approximately 42°C and aseptically add 10ml to each petri dish. Mix the contents by tilting and rotation, allow to solidify as soon as possible after pouring and immediately place in an incubator at 19–21°C. Incubate for 48 ± 3 hours and count at least two plates made from the dilution giving between 30 and 300 colonies per plate.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control

Positive gelatinase:
Serratia liquefaciens ATCC® 27592

Negative gelatinase:
Escherichia coli ATCC® 25922

Precautions

Do not shake the gelatin tubes whilst they are warm because growth and liquefaction of gelatin frequently occurs on the surface layer only[7].

In routine diagnostic work report gelatin liquefaction or not. The type or shape of liquefaction is of less importance[2].

References

1 American Public Health Association (1946) *Standard Methods for the Examination of Water and Sewage. 9th Edn. APHA Inc. Washington DC.*

2 American Society for Microbiology (1981) *Manual of Methods for General Bacteriology. ASM. Washington DC.*

3 Cowan S. T. and Steel K. J. (1966) *Manual for the Identification of Medical Bacteria. Cambridge University Press. Cambridge. pp. 19. 27–28, 116 and 156.*

4 Wilson G. S. and Miles A. A (1964) *Topley and Wilson's Principles of Bacteriology and Immunity. 5th Edn. Vol.1. Edward Arnold. London. pp. 493–494.*

5 Windle Taylor. E. (1958) *'The Examination of Waters and Water Supplies' 7th ed., Churchill Ltd., London, pp. 414 and 422.*

6 Stone R. V. (1935) *Proc. Soc. Exper. Biol. Med. 185–187.*

7 Frobisher M. (1957) *Fundamentals of Microbiology. 6th Edn. W. B. Saunders. Philadelphia. p. 39.*

ORANGE SERUM AGAR

Code: CM657

A medium for the isolation and enumeration of spoilage organisms of citrus products.

Formula	gm/litre
Tryptone	10.0
Yeast extract	3.0
Orange serum (equivalent solids)	3.5
Glucose	4.0
Di-potassium phosphate	2.5
Agar	14.0
pH 5.5 ± 0.2	

Directions
Suspend 37 grams in 1 litre of distilled water and bring gently to the boil to dissolve completely. Dispense into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
Orange Serum Agar was developed specifically for the isolation and enumeration of micro-organisms that are capable of surviving in citrus products[1]. The low pH of these products limits the growth of micro-organisms to those capable of tolerating the acidic environment.

Organisms known to grow in single strength and concentrated citrus juices are lactic acid and acetic acid bacteria, yeasts and moulds[2]. Of this group of organisms lactic acid bacteria have been mainly implicated as the cause of spoilage and these have been identified as *Lactobacillus plantarum* var. *moblis, Lactobacillus brevis, Leuconostoc mesenteroides* and *Leuconostoc dextranicum*[3].

In comparative studies carried out by Murdock et al.[4] Orange Serum Agar was found to be a superior medium when compared to other media used for the total count and detection of spoilage organisms.

Technique
1 Prepare the medium as directed.
2 Add 1ml of the test sample to a sterile petri dish.
3 Add approximately 20ml of cooled medium (50°C) to each plate and mix according to the plate count method[5].
4 Incubate at 30°C and examine daily for up to seven days.
5 Report as number of colony-forming units per ml of test material.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Lactobacillus fermentans ATCC® 9338
Saccharomyces cerevisiae ATCC®9763

Negative control:
Uninoculated medium

Precautions
Do not overheat the medium.

References

1 Hays G. L. (1951) *Proc. Florida State Hort. Soc., 64th Ann. Meeting.*
2 Murdock D. I. and Brokaw C. H. (1958) *Food Tech. 12. 573–576.*
3 Hays G. L. and Riester D. W. (1952) *Food Tech. 6. 386–389.*
4 Murdock D. I., Folinazzo J. F. and Troy V. S. (1952) *Food Tech. 6. 181–185.*
5 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.*

OXYTETRACYCLINE-GLUCOSE-YEAST EXTRACT AGAR (OGYE AGAR)

Code: CM545

For the selective enumeration of moulds and yeasts.

Formula	gm/litre
Yeast extract	5.0
Glucose	20.0
Biotin	0.0001
Agar	12.0
pH 7.0 ± 0.2	

OXYTETRACYCLINE GYE SELECTIVE SUPPLEMENT

Code: SR73

Vial contents (each vial is sufficient for 500ml of medium)
Oxytetracycline (in buffered base) 50mg

NB: When re-constituted the resultant solution is photo-sensitive. It is recommended the solution is added immediately to the prepared agar base. Failure to do so may result in the solution becoming cloudy.

Directions
Suspend 18.5g in 500ml of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 115°C for 10 minutes. Allow the medium to cool to 50°C and aseptically add the contents of one vial of Oxytetracycline GYE Selective Supplement SR73 reconstituted as directed. Mix thoroughly and pour into sterile petri dishes.

Description
Oxytetracycline-Glucose-Yeast Extract Agar is recommended for the selection and enumeration of yeasts and moulds from foodstuffs[1,2]. The medium using oxytetracycline as the selective agent is based on the formulation developed by Mossel et al.[3], who stated that the use of this antibiotic in a medium with a neutral pH gave increased counts of yeasts and moulds from a variety of foodstuffs compared with media which relied on a low pH to suppress bacterial growth. Physically stressed yeast cells give higher counts on media which depend upon broad-spectrum antibiotics rather than a low pH for selectivity[4]. In earlier work Mossel[5] found that Glucose Yeast Extract Agar was as favourable a basal medium as 'Mycophil' Agar later recommended by Sharf[6]. Addition of the oxytetracycline was found to make the Glucose Yeast

Extract Agar more selective than 'Mycophil' Agar by inhibiting the growth of lactobacilli, most of which grow at the acid pH of the latter medium.

The choice of a suitable medium for enumeration of yeasts and moulds is greatly dependent on the nature of the foodstuffs under investigation and the organisms that occur on them[7]. Oxytetracycline-Glucose-Yeast Extract Agar remains bacteriostatic when inoculated with not greater than 1ml of a 10^{-1} dilution of foods and subsequently incubated for not greater than 5 days at 25°C as is the customary practice in food mycology[2].

For isolation of psychotrophic yeasts from chilled proteinaceous foods a combination of oxytetracycline and gentamicin is effective[8].

Very proteinaceous foods and the higher incubation temperatures around 35°C required for some organisms will inactivate oxytetracycline allowing growth of Gram positive and Gram negative rods. For such applications Rose-Bengal Chloramphenicol Agar CM549 may be substituted or Dichloran-Glycerol (DG18) Agar Base CM729.

Technique
Transfer 1ml aliquots of a series of suitable dilutions of the sample to empty 9cm diameter petri dishes. Two dishes are used for each of the dilutions. Add approximately 15ml of medium prepared as described. Mix gently, turning the plates three times clockwise and three times counter-clockwise.

Incubate for 5 days at 22°C ±2 with the petri dishes upside down, checking for formation of aerial mycelia after 2 days.

Count the numbers of colonies in plates containing 50–100 colonies after 5 days, or in any countable plates when aerial mycelia threaten to obscure further readings after 2 days. The counts obtained for each dilution should be similar on both plates.

Calculate the number of yeasts or moulds per 1g or 1ml by multiplying the number of colonies by the dilution factor.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Aspergillus niger ATCC® 9642
Saccharomyces cerevisiae ATCC® 9763

Negative control:
Escherichia coli ATCC® 25922

Precautions:
The lactic-acid bacteria are inhibited on this medium.

References

1 Mossel D. A. A., Harrewijn G. A. and Elzebroek J. M. (1973) *UNICEF.*
2 Mossel D. A. A., Kleynen-Semmeling A. M. C., Vincentie H. M., Beerens H. and Catsaras M. (1970) *J. Appl. Bact. 33. 454–457.*
3 Mossel D. A. A., Visser M. and Mengerink W. H. J. (1962) *Lab. Prac. II, 109–112.*
4 Koburger J. A. and Mace F. E. (1967) *Proc. W. Va. Acad. Sci. 39. 102–106.*
5 Mossel D. A. A. (1951) *Antonie Van Leeuwenhoek 17. 146.*
6 Sharf J. M. (1960) *Ann. Inst. Pasteur, Lille II. 117.*
7 Mossel D. A. A., Vega Clara L. and Put H. M. C. (1975) *J. Appl. Bact. 39. 15–22.*
8 Dijkmann K.E., Koopmans M. and Mossel D.A.A. (1979) *J. Appl. Bact.* 47. ix.

PEPTONE WATER

Code: CM9

A basal medium to which carbohydrates and indicator may be added for fermentation studies.

Formula	gm/litre
Peptone	10.0
Sodium chloride	5.0
pH 7.2 ± 0.2	

Directions
Dissolve 15g in 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

When sterile solutions are to be added after autoclaving, reduce the volume of water for reconstitution by an equal amount.

Description
Peptone Water may be used as a growth medium or as the basis of carbohydrate fermentation media, whilst a pure culture in Peptone Water is a convenient inoculum for a series of fermentation tubes or other diagnostic media.

Peptone Water, adjusted to pH 8.4, is suitable for the cultivation and enrichment of *Vibrio cholerae* from infected material[1].

The medium was formerly used for the performance of the indole test, but now better results can be obtained by the use of Tryptone Water CM87.

Peptone Water may be modified to make it suitable for carbohydrate fermentation tests by the addition of Andrade indicator and the required carbohydrate, (Peptone Water sugars).

Andrade indicator is a solution of acid-fuchsin tritrated with sodium hydroxide until the colour changes from pink to yellow. When the indicator is added to Peptone Water it is colourless to slightly pink at pH 7.2, becomes pink at acid pH levels and yellow at alkaline pH levels (range 5–8). Filter-sterilised 'sugar' solutions are added to the base medium after sterilisation. These solutions are usually at 10% w/v concentrations and it is important to allow for dilution of the Peptone Water when making up the initial volume of medium. A final concentration of 1% w/v sugar in Peptone Water is normally used but more expensive sugar can be used at 0.5%.

Andrade's indicator may be made by adding 1 *N* sodium hydroxide to a 0.5% solution of fuchsin until the colour just becomes yellow.

Appropriate safety precautions must be taken to avoid inhalation of, and skin contact with, acid fuchsin.

Both Peptone Water and Andrade Peptone Water are prepared and sterilised in the same manner except that an inverted fermentation tube (Durham tube) to detect gas production is included in Andrade Peptone Water containing glucose. Some organisms will utilise carbohydrate to produce acid only without gas formation. It is unnecessary to add Durham tubes to Peptone Water sugars other than glucose.

Precautions for Andrade Peptone Water sugars
Make sure that each individual bottle of Peptone Water sugar is correctly coded for the contained sugar.

Andrade Peptone Water is reddish-pink when hot; it should return to a colourless or a slightly pink colour when cooled to room temperature.

Some sugar solutions may affect the pH of the Peptone Water; check and correct if so.

Sub-cultures may be necessary to ensure purity of the inoculant. Mixed or contaminated cultures will give false reactions.

Andrade indicator will fade on prolonged storage; do not use beyond the expiry date.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Maintain stock cultures of organisms which have known positive and negative reactions in each sugar. Using fresh sub-cultures, test each batch of sugar media with the appropriate organisms.

Reference
1 Cruickshank R. (1968) *'Medical Microbiology' 11th ed., Livingstone Ltd., London, p. 268.*

PERFRINGENS AGAR (OPSP)

Code: CM543

For the enumeration of Cl. perfringens in foods.

Formula	gm/litre
Tryptone	15.0
Yeast extract	5.0
Soya peptone	5.0
Liver extract	7.0
Ferric ammonium citrate	1.0
Sodium metabisulphite	1.0
Tris buffer	1.5
Agar	10.0
pH 7.3 ± 0.2	

PERFRINGENS (OPSP) SELECTIVE SUPPLEMENT A

Code: SR76

Vial contents (each vial is sufficient for 500ml of medium)

Sodium sulphadiazine	50mg

PEFRINGENS (OPSP) SELECTIVE SUPPLEMENT B

Code: SR77

Vial contents (each vial is sufficient for 500ml of medium)

Oleandomycin phosphate	0.25mg
Polymyxin B	5000 IU

Directions
Suspend 22.8g in 500ml of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Allow to cool to 50°C and aseptically add the contents of one vial each of Perfringens Agar (OPSP) supplements A and B SR76 and SR77 which have been rehydrated by the addition of 2ml of sterile distilled water. Mix well and pour into sterile dishes.

Description
Oxoid Perfringens Agar (OPSP) CM543, is based on the formulation developed by Handford[1].

The medium utilises sulphadiazine (100μg/ml), oleandomycin phosphate (0.5μg/ml) and polymyxin B sulphate (10IU/ml), presented as freeze-dried supplements SR76 and SR77 to give a high degree of selectivity and specificity for *Clostridium perfringens.*

Sodium metabisulphite and ammonium ferric citrate are used as an indicator of sulphite reduction by *Cl. perfringens* which produces black colonies on this medium when using a pour plate technique.

Tests for confirmation of *Cl. perfringens* are described in a study initiated by the International Commission on Microbiological Specifications for Foods (I.C.M.S.F.)[2].

Sulphite reducing bacteria other than *Cl. perfringens* such as salmonellae, *Proteus* species and *Citrobacter freundii*, as well as staphylococci and *Bacillus* species, are inhibited on OPSP Agar.

Perfringens Agar (OPSP) has the advantage of inhibiting growth of *Cl. bifermentans* and *Cl. butyricum.* These sulphite reducing organisms grow readily on Shahidi-Ferguson Perfringens Agar (SFP)[3] and Tryptone-Sulphite-Neomycin Agar (TSN)[4] as black colonies with a tendency to spread and obscure the whole surface of the medium.

Occasional strains of enterococci will grow on Perfringens Agar (OPSP) as white colonies, easily distinguished from the large black colonies of *Cl. perfringens.*

Cl. perfringens enumeration media which include egg yolk in order to detect lecithinase activity have not proved satisfactory partly because *Cl. perfringens* colonies may frequently fail to produce haloes and thus appear falsely to be negative, and partly because counting is rendered impractical as the organism often grows in the form of large spreading colonies which completely blacken the medium[5].

Technique
Make up the medium according to the directions. Prepare pour plates, containing approximately 25ml per plate, using 1ml aliquots of a suitable series of dilutions of the homogenised test sample. Mix well before setting.

It is unlikely that colonies of *Cl. perfringens* will blacken if plates are surface-inoculated unless the inoculum is covered with a layer of agar.

Incubate the plates at 35°C for 18–24 hours with a H_2/CO_2 Gas Generating Kit pack BR38 in a conventional gas-jar. Alternatively use Anaerogen AN025A or AN035A. Anaerogen does not require the addition of water or a catalyst.

Cl. perfringens may be seen as large black colonies (2–4mm diameter) within the depth of the agar.

Occasional strains of *Enterococcus faecalis* which may grow on Perfringens Agar (OPSP) as small colourless colonies are easily distinguished from *Cl. perfringens*.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Clostridium perfringens ATCC® 13124

Negative control:
Clostridium bifermentans ATCC® 638

Precautions
The production of black colonies on this medium is a presumptive identification of *Cl. perfringens*. Further identification tests must be carried out.

References

1 Handford P. M. (1974) *J. Appl. Bact. 37. 559–570.*
2 Hauschild A. H. W., Gilbert R. J., Harmon S. M., O'Keeffe M. F. and Vahlefeld R. (1977) *ICMSF Methods Studies VIII, Can. J. Microbiol. 23. 884–892.*
3 Shahidi S. A. and Ferguson A. R. (1971) *Appl. Microbiol. 21. 500–506.*
4 Marshall R. S., Steenbergen J. F. and McClung L. S. (1965) *Appl. Microbiol. 13. 559–562.*
5 Hauschild A. H. W. and Hilsheimer R. (1974) *Appl. Microbiol. 27. 78–82.*

PERFRINGENS AGAR BASE (TSC AND SFP)

Code: CM587

A basal medium for use with selective agents to make either TSC agar or SFP agar for the presumptive identification and enumeration of Clostridium perfringens.

Formula	gm/litre
Tryptose	15.0
Soya peptone	5.0
'Lab-Lemco' powder	5.0
Yeast extract	5.0
Sodium metabisulphite	1.0
Ferric ammonium citrate	1.0
Agar	14.0
Final pH 7.6 ± 0.2	

PERFRINGENS (SFP) SELECTIVE SUPPLEMENT

Code: SR93

Vial contents (each vial is sufficient for 500ml of medium)

Kanamycin sulphate	6.mg
Polymyxin B	15000 IU

PERFRINGENS (TSC) SELECTIVE SUPPLEMENT B

Code: SR88

Vial contents (each vial is sufficient for 500ml of medium)

D-cycloserine	200mg

Directions

To Prepare the Agar Base

Suspend 23g in 500ml of distilled water and heat gently until the agar is completely dissolved. Sterilise by autoclaving at 121°C for 10 minutes. Allow the medium to cool to 50°C.

To Prepare Tryptose Sulphite Cycloserine Agar (TSC Agar)

To 500ml of Agar base cooled to 50°C add the rehydrated contents of 1 vial of TSC supplement, SR88 and 25ml of egg yolk emulsion, SR47. Mix well and pour into sterile petri dishes.

To Prepare Egg Yolk Free TSC Agar

To 500ml of Agar base cooled to 50°C add the rehydrated contents of 1 vial of TSC supplement SR88. Mix well and pour into sterile petri dishes.

To Prepare Shahidi-Ferguson Perfringens Agar (SFP Agar)

To 500ml of Agar base cooled to 50°C add the rehydrated contents of 1 vial of SFP supplement SR93 and 25ml of egg yolk emulsion SR47, mix well and pour into sterile petri dishes.

To Prepare Agar for an Overlay

For TSC or SFP Agar used as an overlay, the egg yolk emulsion, SR47, is omitted. Its inclusion does not improve the lecithinase reaction and diminishes the visibility of the colonies.

Description

Perfringens Agar Base (TSC and SFP) CM587 is a nutrient medium to which is added egg yolk emulsion SR47 and the appropriate antibiotic supplement to prepare either Shahidi-Ferguson Perfringens (SFP) Agar using SR93 or Tryptose Sulphite Cycloserine (TSC) Agar using SR88.

An egg yolk free TSC Agar had been described[4,5] which has the advantage that smaller colonies are formed. This can simplify the counting of plates with high numbers of colonies. Higher counts have been demonstrated by using it with a pour plate technique. The differences were thought to be due to exposure of the *Cl. perfringens* cells to high oxygen tension in the surface plating procedure[4].

Shahidi-Ferguson Perfringens Agar is based on the formulation developed by Shahidi and Ferguson[1]. The medium utilises kanamycin sulphate (12mg/litre) and polymyxin B sulphate (30,000 IU/litre) as the selective agents to give a high degree of selectivity and specificity for *Cl. perfringens*.

Tryptose Sulphite Cycloserine Agar was developed using the same basal medium as SFP Agar[2] but with 400mg/litre of D-cycloserine as the selective agent.

Sodium metabisulphite and ferric ammonium citrate are used as an indicator of sulphite reduction by *Cl. perfringens* which produces black colonies in both media.

Trials[3] have indicated that polymyxin B and kanamycin sulphate used in SFP Agar allow a greater recovery of both vegetative cells and spores of *Cl. perfringens* than either polymyxin B and sulphadiazine used in Sulphite Polymyxin Sulphadiazine Agar, or neomycin, used in Tryptone Sulphite Neomycin Agar. However, a greater number of non-specific colonies were found on SFP Agar.

In another study[2], *Serratia marcescens* and *Streptococcus lactis* were the only faculative anaerobes to grow on TSC Agar, whereas SFP Agar also allowed the growth of *Enterococcus, Proteus* and *Enterobacter* strains, but allowed a slightly higher rate of recovery of *Cl. perfringens* than TSC Agar.

Both SFP Agar and TSC Agar permitted growth of other sulphite-reducing *Clostridium* species tested, with the exception of *Cl. sordellii* which was completely inhibited and *Cl. bifermentans* which was partially inhibited on TSC Agar. Both strains grew on SFP Agar.

Some strains of *Cl. perfringens* may produce an opaque zone around the colony due to lecithinase activity, but this is not considered to be universal for all *Cl. perfringens* strains after overnight incubation[4] and both black lecithinase-positive and black lecithinase-negative colonies should be considered as presumptive *Cl. perfringens* on TSC or SFP Agars and confirmatory tests carried out. Egg yolk positive facultative anaerobes may grow on SFP Agar to produce completely opaque plates thus masking the egg yolk reaction of *Cl. perfringens*.

Technique

1 Make up the medium according to the directions and prepare plates containing approximately 20ml of a basal layer of TSC or SFP Agar containing egg yolk.

2 Prepare 0.1ml aliquots of a suitable series of dilutions of the homogenised test sample and spread over the surface of the basal layer using a sterile swab.

3 Overlay with an additional 10ml of egg yolk free TSC or SFP Agar.

   Cultures which are not overlaid with agar are unlikely to grow as black colonies.

4 Incubate the plates at 35°C for 18–24 hours with an anaerobic Gas Generating Kit, BR38, in a gas-jar. Alternatively use Anaerogen AN025A or AN035A. Anaerogen does not require the addition of water or a catalyst.

Alternatively, pour-plates using approximately 25ml per plate of TSC or SFP Agar containing egg yolk may be prepared using 1ml aliquots of a suitable series of dilutions of the homogenised test sample. Mix the plates well before the agar gels. With this technique, lecithinase activity of *Cl. perfringens* colonies is more difficult to see.

Cl. perfringens colonies may be seen as large, black (2–4mm diameter) colonies within the depth of the agar.

Egg yolk free TSC Agar is used with the techniques described above. *Cl. perfringens* colonies are black but in the absence of egg yolk no lecithinase activity can be detected.

Tests for confirmation are described in a study initiated by the International Commission on Microbiological Specifications for Foods[6] involving nitrate reduction, lactose fermentation, gelatin liquefaction and the absence of motility. All black colonies growing on TSC or SFP Agars should be tested.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Clostridium perfringens ATCC® 13124

Negative control:
Clostridium sordellii ATCC® 9714

Precautions
Black colonies appearing on these two media may be organisms other than *Cl. perfringens*.

References

1 Shahidi S. A. and Ferguson A. R. (1971) *Appl. Microbiol. 21. 500–506.*
2 Harmon S. M., Kauttar D. A. and Peeler J. T. (1971) *Appl. Micobiol. 22. 688–692.*
3 Harmon S. M., Kautter D. A. and Peeler J. T. (1971) *Appl. Microbiol. 21. 922–927.*
4 Hauschild A. H. W. and Hilsheimar R. (1973) *Appl. Microbiol. 27. 78–82.*
5 Hauschild A. H. W. and Hilsheimar R. (1973) *Appl. Microbiol. 27. 521–526.*
6 Hauschild A. H. W., Gilbert R. J., Harmon S. M., O'Keefe M. F. and Vahlfeld R. (1977) *Can. J. Microbiol. 23. 884–892.*

PLATE COUNT AGAR

TRYPTONE GLUCOSE YEAST AGAR

Code: CM325

A medium for the enumeration of viable organisms in milk and dairy products.

Formula	gm/litre
Tryptone	5.0
Yeast extract	2.5
Glucose	1.0
Agar	9.0
pH 7.0 ± 0.2	

Directions
Add 17.5g to 1 litre of distilled water. Dissolve by bringing to the boil with frequent stirring, mix and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Plate Count Agar is equivalent to the medium recommended by the APHA[1] for the plate count of micro-organisms in milk and other dairy products. Basically the APHA method consists of the preparation of pour-plates using diluted samples, and counting colonies after incubation. Incubation is for 48 hours at 32°C or at 35°C for the Standard Plate Count. For the enumeration of micro-organisms with other temperature requirements, plates may also be incubated for 7–10 days at 5–7°C: for 3–5 days at 20°C; for 2–3 days at 45°C; or for 48 hours at 55°C. See the APHA[1] publication for details.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control
Compare with previous lot/batch using pasteurised and raw milk samples, incubated at 32–35°C for 48 hours.

Precautions
Make sure that the procedures and media used in milk product testing comply with the National Regulations required for each country.

Reference

1 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.*

STANDARD PLATE COUNT AGAR (APHA)

Code: CM463

A standard medium corresponding to the APHA formulation for milk, water, food and dairy products.

Formula	gm/litre
Yeast extract	2.5
Pancreatic digest of casein	5.0
Glucose	1.0
Agar	15.0
pH 7.0 ± 0.2	

Directions
Suspend 23.5g in 1 litre of distilled water. Bring to the boil to dissolve completely. Dispense into bottles and sterilise by autoclaving at 121°C for 15 minutes.

Description
Standard Plate Count Agar was developed by Buchbinder et al.[1] who wished to use an agar without milk solids in the formulation and investigated the control tests necessary to give standard results in dairy products with statistically valid counts.

It is prepared from selected ingredients and tested by the APHA protocol[2].

Standard Plate Count Agar meets the prescribed standards of the APHA[3], and AOAC[4].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control
Compare with previous lot/batch using pasteurised and raw milk samples, incubated at 32–35°C for 48 hours.

Precautions
When carrying out prescribed Standard Methods it is essential to follow the protocols given in precise detail.

References

1 Buchbinder et al. (1951) *Public Health Reports. 66. 327–329.*
2 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.*
3 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.*
4 Association of Official Analytical Chemists (1978) *Bacteriological Analytical Manual. 5th Edn. AOAC. Washington DC.*

MILK PLATE COUNT AGAR

PLATE COUNT AGAR WITH ANTIBIOTIC FREE SKIM MILK

Code: CM681

A medium for the enumeration of viable organisms in milk and dairy products.

Formula	gm/litre
Tryptone	5.0
Yeast extract	2.5
Glucose	1.0
Antibiotic free skim milk	1.0
Agar	10.0
pH 7.0 ± 0.1	

Directions
Suspend 19.5g in 1 litre of distilled water. Bring to the boil with frequent agitation, mix and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Plate Count Agar with Antibiotic Free Skim Milk is equivalent to the medium recommended by British Standards Institution[1] and International Organization for Standardization[2]. The medium is used for the enumeration of viable organisms in milk and dairy products.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control
Compare with previous lot/batch using pasteurised and raw milk samples, incubated at 32–35°C for 48 hours.

Precautions
Make sure that the procedures and media used in milk product testing comply with the National Regulations required for each country.

References

1 British Standards Institution BS4285 Section 1.2 1984.
2 International Organization for Standardization. Draft International Standard ISO/DIS 6610 : 1982.

POTATO DEXTROSE AGAR

Code: CM139

A medium recommended for the detection and enumeration of yeasts and moulds in butter and other dairy and food products.

Formula	gm/litre
Potato extract	4.0
Glucose	20.0
Agar	15.0
pH 5.6 ± 0.2	

Directions
Suspend 39g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring.

In order to suppress bacterial growth it is sometimes desirable to acidify the medium to pH 3.5. This can be done by adding 1ml of Lactic Acid 10% SR21 to each 100ml of sterilised medium at 50°C. The medium must not be heated after the addition of the acid, this would result in hydrolysis of the agar and destroy its gelling properties.

Description
A suitable medium for the isolation and count of yeasts and moulds in butter etc.[1] or those occurring on the surface of fresh meats, cured meats and sausage products[2].

Work carried out in cooperation with CSIRO Melbourne had shown that the minerals present in agar could influence the pigment formation of certain fungi. Where pigment production is a critical part of the identification of the fungus it is clearly important to stabilise this characteristic. The agar used in Potato Dextrose Agar is carefully screened to ensure correct pigment production by fungi such as *Fusaria* species.

Technique
After sterilising the reconstituted medium adjust the reaction to pH 3.5 by adding 1ml of Lactic Acid 10% SR21 to each 100ml of medium at 50°C. Do not reheat after acidification.

Prepare dilute emulsions or suspensions of the product to be tested, make pour-plates in the usual manner, and incubate for 5 days at 21°C. Count the number of yeast and mould colonies.

If a non-selective medium is required, it is suggested that Potato Dextrose Agar may be used without added acid or, alternatively, one may use a general purpose mycological medium such as Malt Extract Agar CM59.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Aspergillus niger ATCC® 9642

Negative control:
Uninoculated medium or if at pH 3.5 *Bacillus subtilis* ATCC® 6633.

References

1 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.*
2 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.*

PSEUDOMONAS AGAR BASE

Code: CM559

For the selective isolation of Pseudomonas species when supplemented with SR102 or SR103.

Formula	gm/litre
Gelatin peptone	16.0
Casein hydrolysate	10.0
Potassium sulphate	10.0
Magnesium chloride	1.4
Agar	11.0
pH 7.1 ± 0.2	

CN SELECTIVE SUPPLEMENT

Code: SR102

Vial contents (each vial is sufficient for 500mls of medium)

Cetrimide	100mg
Sodium nalidixate	7.5mg

CFC SELECTIVE AGAR SUPPLEMENT

Code: SR103

Vial contents (each vial is sufficient for 500mls of medium)

Cetrimide	5.0mg
Fucidin	5.0mg
Cephaloridine	25.0mg

Directions
To Prepare the Agar Base
Suspend 24.2g of the agar base, CM559, in 500ml of distilled water. Add 5ml of glycerol. Bring to the boil to dissolve completely, sterilise by autoclaving at 121°C for 15 minutes. Allow the medium to cool to 50°C.

To Prepare Pseudomonas C-N Agar
To 500ml of agar base cooled to 50°C add the contents of 1 vial of Pseudomonas C-N Supplement SR102 rehydrated with 2ml of sterile distilled water/ethanol (1:1). Mix well and pour into sterile petri dishes.

To Prepare Pseudomonas C-F-C Agar
To 500ml of agar base cooled to 50°C add the contents of 1 vial of Pseudomonas C-F-C Supplement SR103 rehydrated with 2ml of sterile distilled water/ethanol (1:1). Mix well and pour into sterile petri dishes.

Description
Pseudomonas Agar Base CM559 is designed so that by the addition of the appropriate supplement, SR102 or SR103, the medium becomes selective for *Ps. aeruginosa* or *Pseudomonas* spp. generally. The base medium is a modification of King's A Medium[1] in which magnesium chloride and potassium sulphate are present to enhance pigment production.

Pseudomonas C-N Supplement SR102 is recommended for the selective isolation of *Ps. aeruginosa*. The formula of the supplement was described by Goto and Enomoto[2] following the demonstration of cetrimide as a selective agent by Lowbury and Collins[3]. Goto and Enomoto showed that addition of nalidixic acid at 15µg/ml, while at the same time reducing the cetrimide content to 200µg, improved performance. The medium gave better recovery of *Ps. aeruginosa* with enhanced pigment formation whilst strongly suppressing *Klebsiella, Proteus* and *Providencia* spp., the latter organisms being the troublesome contaminants of conventional cetrimide media.

Pseudomonas C-F-C Supplement SR103 is recommended for the selective isolation of *Pseudomonas* spp. generally. Mead and Adams[4] showed that reducing the cetrimide content to 10µg/ml allowed the growth of all pigmented and non-pigmented psychrophilic pseudomonads. To improve its selective action they added cephaloridine 50µg/ml) and fucidin (10µg/ml). This combination of agents gave a new and more specific medium for isolating pseudomonads from chilled foods and processing plants. Incubation should be carried out at 25–30°C for 48 hours.

Considerable importance is given to detection of *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) in water systems, particularly where the water is to be used for the preparation of pharmaceuticals and cosmetics[5]. The organism is resistant to many commonly-used disinfectants. *B. cepacia* has emerged as an important opportunistic pathogen in urinary, abdominal, respiratory and other infections.

Growth characteristics of *Pseudomonas* species on Oxoid Pseudomonas Agar Base with Supplements.

	Amount of growth on	
***Pseudomonas* species**	**Supplement C-N (SR102)**	**Supplement C-F-C (SR103)**
B. cepacia NCTC 10661	±	+++
B. cepacia NCTC 10743	+	+++
P. aeruginosa	+++	+++
P. putida	++	+++
P. fluorescens	+++	+++

Incubation carried out at 30°C and plates read after 18 hours incubation.

Technique
Clinical Specimens for Ps. aeruginosa Investigations
1 Prepare Pseudomonas C-N Medium as directed.
2 Pour plates and dry the surface.

3 Swab a large inoculum over half the area of the plate.
4 Using a sterile loop, streak out the inoculum over the remainder of the plate to obtain isolated colonies.
5 Incubate at 35°C and examine after 24 and 48 hours, using both white and ultraviolet light.

Food, Water and Environmental Samples for Pseudomonads

1 Prepare Pseudomonas C-F-C Medium as directed.
2 Pour plates and dry the surface.
3 Prepare food samples by diluting 1 in 5 or 1 in 10 with 1% (w/v) sterile Peptone Water, CM9, and homogenise in a 'Stomacher' or a laboratory blender.
4 Pipette 0.5 or 1ml of the homogenate on to the plate and spread over the surface with a sterile glass spreader. Inoculate water and swab samples directly on the surface of the medium.
5 Incubate at 25°C and examine after 24 and 48 hours, using both white and ultraviolet light.

Colonial Appearance

Growth on C-N or C-F-C Medium is usually limited to *Pseudomonas* spp. but some members of the family Enterobacteriaceae may also be present. The presence of blue-green or brown pigmentation, or fluorescence may be taken as presumptive evidence of *Pseudomonas* spp. but further tests must be carried out to confirm the identity of the organism.

Stanbridge and Board[6] modified C-F-C Medium to differentiate pseudomonads from Enterobacteriaceae developing on beef steaks packaged in modified atmospheres. Arginine 1% w/v and phenol red 0.002% w/v were added to the medium.

Pseudomonads produce ammonia from the arginine and colonies may be distinguished by a pink coloration.

Storage conditions and Shelf life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control

C-N formulation

Positive control:
Pseudomonas aeruginosa ATCC® 27853

Negative control:
Proteus vulgaris ATCC® 13315

C-F-C formulation

Positive control:
Burkholderia cepacia NCTC 10661

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions

Fresh media should be prepared as required. Molten agar should not be kept longer than 4 hours. Medium should not be stored and remelted. If swarming colonies of *Proteus* species are a problem in food samples then the incubation temperature can be lowered to 20°C for a period of 3–5 days. Chilled foods may carry a wide range of pseudomonads and the colonies on C-F-C Medium, incubated at lower temperatures, may be *Ps. fluorescens* or *Ps. putida* as well as *Ps. aeruginosa*. *Aeromonas* species will also appear as pink/brown colonies, particularly from fish products.

References

1 King E.O., Ward M.K. and Raney D.E. (1954) *J. Lab. & Clin. Med. 44. 301–307.*
2 Goto S. and Enomoto S. (1970) *Jap. J. Microbiol. 14. 65–72.*
3 Lowbury E.J. and Collins A.G. (1955) *J. Clin. Path. 8. 47–48.*
4 Mead G.C. and Adams B.W. (1977) *Br. Poult. Sci. 18. 661–667.*
5 Geftic S.G., Heymann H. and Adair F.W. (1970) *App. & Environmental Microbiol. 37. 505–510.*
6 Stanbridge L.H. and Board R.G. (1994) *Lett. Appl. Microbiol. 18. 327–328.*

R2A AGAR

Code: CM906

A medium for the bacterial examination of drinking water.

Formula	gm/litre
Yeast extract	0.5
Tryptone	0.25
Peptone	0.75
Dextrose	0.5
Starch	0.5
Di-potassium phosphate	0.3
Magnesium sulphate	0.024
Sodium pyruvate	0.3
Agar	15.0
pH 7.2 $\pm$ 0.2	

Directions
Suspend 18.1 grams of Agar CM906 in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Standard methods for enumeration of heterotrophic bacteria in water utilise nutritionally rich media, and incubation at 35°C. Organisms isolated under these conditions may represent only a small percentage of the bacteria present in the sample.

R2A Agar used at lower incubation temperatures, over a longer period will recover some organisms, which are stressed or chlorine tolerant, leading to a more realistic estimate of bacterial numbers.

Technique
R2A Agar may be used in poured plate, spread plate and membrane filtration procedures. Standard methods should be followed for sample collection and testing.

Recommended incubation is for 5–7 days at 20°C or 28°C, or for 3 days at 35°C.

Storage conditions and Shelf life
R2A Agar should be stored tightly capped in the original container at 10°C to 25°C. When stored as directed the medium will remain stable until the expiry date printed on the label.

The prepared medium may be stored for up to 2 weeks at 2–8°C.

Quality Control
R2A Agar may be tested for performance using stable, typical control cultures.

References

1 Collins and Willoughby (1962) *Arch. Microbiol. 43, 294.*
2 Greenbreg, Trussell and Clesceri (ed) *(1958) Standard Methods for the Examination of Drinking Water and Waste Water. 16th Ed. APHA, Washington DC.*
3 Reasoner and Geldreich (1985). *Appl. Environ. Microbiol. 49, 1.*
4 Stark and McCoy (1938) *Zentrabl. Bakterio: Infektionskr. Hyg. Abt. 2. 89, 201.*

RAKA-RAY AGAR

Code: CM777

The addition of phenylethanol and cycloheximide forms a selective medium for the isolation of lactic acid bacteria in beer and brewing processes.

Formula	gm/litre
Yeast extract	5.0
Tryptone	20.0
Liver concentrate	1.0
Maltose	10.0
Fructose	5.0
Glucose	5.0
Betaine HCL	2.0
Diammonium hydrogen-citrate	2.0
Potassium aspartate	2.5
Potassium glutamate	2.5
Magnesium sulphate. $7H_2O$	2.0
Manganese sulphate. $4H_2O$	0.66
Potassium phosphate	2.0
N-acetyl glucosamine	0.5
Agar	17.0
pH 5.4 $\pm$ 0.2	

Supplement	per litre
Sorbitan mono-oleate	10ml
Cycloheximide	7mg
2-Phenylethanol	3g

Directions
Suspend 77.1 grams in 1 litre of distilled water. Add 10ml of sorbitan mono-oleate and 7mg of cycloheximide. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50–55°C and aseptically add 3 grams of phenylethanol. Pour into sterile petri dishes or distribute into 4ml volumes held at 55°C if the overlay technique is to be used.

Description
Raka-Ray Agar, CM777, is based on the formula of Saha, Sondag and Middlekauff for the detection of lactic acid bacteria in beer and brewing processes[1]. Its use is recommended by the American Society of Brewing Chemists (ASBC)[2], and the European Brewing Convention (EBC)[3].

Members of the family Lactobacillaceae occurring in the brewing process are important spoilage organisms because products arising from their growth and metabolism are often seriously detrimental to flavour. Detection is complicated by the diverse nutritional and environmental requirements of the family and a considerable number of formulations have been described arising from attempts to optimise conditions.

Raka-Ray 3 Medium[1] was developed to enable brewers to monitor in-process beer quickly and accurately for a wide range of organisms including pediococci.

Investigations in which various combinations of growth stimulating agents were added to Universal Beer Agar led to the recognition of a number of agents including sorbitan mono-oleate, liver extract, yeast extract and N-acetyl glucosamine which gave superior results in respect of colony size, colony

numbers and incubation time when compared with unmodified Universal Beer Agar.

These investigations provided the basis for the formula of Raka-Ray 3 Medium in which sorbitan mono-oleate is included as a stimulant for lactic acid bacteria in general[4]. Fructose is present as the essential carbohydrate source for *Lactobacillus fructivorans*[5] while maltose is present to detect lactobacilli which cannot utilise glucose[6].

Detailed changes to the published Raka-Ray 3 formula are common, arising from attempts to further improve the performance for particular organisms and strains. Pediococci appear to have a universal ability to utilise glucose[7]. The value of partial substitution by glucose of the fructose content has been noted.

Selectivity is achieved by the addition of 3gm/litre of 2-phenylethanol to inhibit Gram-negative organisms and 7mg of cycloheximide to inhibit yeasts[8].

In a review of the performance of various media, Van Keer et al.[5] found that Raka-Ray 3 yielded the highest colony count and allowed the enumeration of the greatest number of strains within 48 hours from a total of 30 strains of *Lactobacillus* taken from different origins and incubated under semi-anaerobic conditions.

Hsu and Taparowsky[9], when comparing Raka-Ray 3 and MRS Agar found the Raka-Ray formulation to be superior for *Pediococcus cerevisiae* although it was not as efficient for *L. gayonii*. In another study Hug, Schlienger and Pfenniger[10] compared Raka-Ray 3 with a number of other lactobacillus media including MRS and sucrose agars and concluded that Raka-Ray 3 and MRS were the best.

Technique

Surface Inoculation

0.1ml of the sample is spread on agar plates. Incubate at 25–30°C under anaerobic conditions using the Oxoid Gas Generating Kit BR38 with the Oxoid Anaerobic Jar. Alternatively, the specimen can be filtered and the membrane placed on the agar surface for incubation.

Overlay Technique

Aseptically dispense 4ml volumes of Raka-Ray Agar into small test tubes and keep molten in a water bath at 55°C.

Mix 1ml of the test sample with 4ml of molten agar and immediately pour the contents into a petri dish containing 15–20ml of solid Raka-Ray Agar to give well distributed colonies. Incubate under anaerobic conditions at 25–30°C in an Oxoid Anaerobic Jar with a Gas Generating Kit BR38. Alternatively use AnaeroGen AN025A or AN035A. AnaeroGen does not require the addition of water or a catalyst.

Because the agar layer is very thin, individual colonies can be picked easily for further examination.

Incubation Conditions

An incubation period of 4 days is generally sufficient but slower growing organisms may require up to 7 days.

Because of the diversity of environmental conditions required for growth of lactic acid bacteria a semi-anaerobic atmosphere may be needed. This is achieved using Oxoid Gas Generating Kit BR56 in the Oxoid Anaerobic Jar. Alternatively use CampyGen CN025A or CN035A. CampyGen does not require the addition of water or a catalyst.

Storage conditions and Shelf life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control

Positive control:
Lactobacillus fermentans ATCC® 9338

Negative control:
Escherichia coli ATCC® 25922

Precautions

Although the concentration of cycloheximide in the medium is below toxic levels, precautions should be observed as detailed under HAZARDS page 2–7.

References

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RAPPAPORT-VASSILIADIS (RV) ENRICHMENT BROTH

Code: CM669

A selective enrichment broth for the isolation of salmonellae.

Formula (Classical)	gm/litre
Soya peptone	5.0
Sodium chloride	8.0
Potassium dihydrogen phosphate	1.6
Magnesium chloride $6H_2O$	40.0
Malachite green	0.04
pH 5.2 ± 0.2	

THIS MEDIUM IS VERY HYGROSCOPIC AND MUST BE PROTECTED FROM MOISTURE.

The quantities given for the formula as classically described made 1110ml of medium. They have been published this way in the Oxoid literature to coincide with the scientific literature.

The directions for reconstituting Oxoid Rappaport-Vassiliadis (RV) Enrichment Broth CM669 follow

usual Oxoid practice and specify the weight needed for 1 litre of medium.

Directions
Add 30g (the equivalent weight of dehydrated medium per litre) to 1 litre of distilled water. Heat gently until dissolved completely. Dispense 10ml volumes into screw-capped bottles or tubes and sterilise by autoclaving at 115°C for 15 minutes.

Description
Rappaport-Vassiliadis (RV) Enrichment Broth CM669 is based on the formulation described by van Schothorst and Renaud[1] and is recommended as the selective enrichment medium when isolating *Salmonella* species from food and environmental specimens. It can also be used to isolate salmonella from human faeces without pre-enrichment but the inoculum must be small. The original formulation described by Rappaport et al.[2] was specifically developed to exploit the four characteristics of *Salmonella* species when compared with other Enterobacteriaceae.

1 The ability to survive at relatively high osmotic pressures.
2 To multiply at relatively low pH values.
3 To be relatively more resistant to malachite green.
4 To have relatively less demanding nutritional requirements.

Oxoid's Rappaport-Vassiliadis (RV) Enrichment Broth is similar to that described by Vassiliadis et al.[3] except that the peptone used is soya peptone, which has been reported to enhance the growth of salmonellae[1,11].

Rappaport Broth was found[2] to be superior to Selenite Enrichment Broth and Tetrathionate Broth for enrichment of salmonellae with the exception of *S. typhi.* Vassiliadis et al.[3] modified Rappaport Broth by lowering the concentration of malachite green and raising the incubation temperature to 43°C. This modified Rappaport Enrichment Broth is RV or Rappaport-Vassiliadis Medium and has been found to be superior to other salmonella selective enrichment media, especially when small inocula of pre-enrichment broth are used[4,5,6,7,8].

In an evaluation of different enrichment media for isolation of salmonella from seawater, Rappaport-Vassiliadis (RV) Broth and the same broth supplemented with novobiocin were the best for detection and enumeration of salmonellae in samples with low and moderate pollution levels[9].

In another study[10], Rappaport-Vassiliadis (RV) Broth was found to be superior to tetrathionate-brilliant green broth for the detection of salmonellae in artificially contaminated fluid whole milk.

It is important that the inoculum size used for enrichment culture in RV Broth is sufficiently small not to interfere with its selectivity. Inoculum/broth ratios 1:100 to 1:2000 have been suggested[12].

Technique
Food and Environmental Specimens
1 Prepare Buffered Peptone Water (Oxoid CM509) as directed in containers containing 225ml of the medium.
2 Prepare Rappaport-Vassiliadis (RV) Enrichment Broth CM669 as directed.
3 Add 25g of the test specimen to 225ml of Buffered Peptone Water and incubate at 35°C for 16–20 hours.
4 Inoculate 0.1ml of the pre-enrichment peptone water culture to 10ml of Rappaport-Vassiliadis (RV) Enrichment Broth and incubate at 42°C 1°C for 24–48 hours.*
5 Sub-culture the broth by streaking on to plates of Brilliant Green Agar (Modified) CM329. Incubate at 35°C for 18–24 hours.
6 Colonies showing typical Salmonella colonial morphology should be confirmed by biochemical or serological methods.

* The recommended incubation temperature is 43°C but this is a critical upper limit. To allow for incubator temperature fluctuation 42°C ± 1°C is a preferred recommendation with 42°C ± 0.1°C for water baths.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922

Precautions
RV Broth should not be used if *Salm. typhi* is suspected.

Note the difference in weight between the classical formula on the label and the reduced weight per litre, using anhydrous magnesium chloride.

References

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RAPPAPORT-VASSILIADIS SOYA (RVS) PEPTONE BROTH

Code: CM866

A selective enrichment broth for the isolation of salmonellae.

Formula	gm/litre
Soya peptone	4.5
Sodium chloride	7.2
Potassium dihydrogen phosphate	1.26
Di-potassium hydrogen phosphate	0.18
Magnesium chloride (anhydrous)	13.58
Malachite green	0.036
pH 5.2 ± 0.2	

Directions
Suspend 26.75 grams in 1 litre of distilled water and heat gently to dissolve. Dispense 10ml volumes into screw-capped bottles or tubes and sterilise by autoclaving at 115°C for 15 minutes.

Description
Rappaport-Vassiliadis Soya (RVS) Peptone Broth is recommended as a selective enrichment medium for the isolation of Salmonellae from food and environmental specimens.

RVS Broth CM866 shares with the original formulation[1], the ability to exploit the full characteristics of *Salmonella* species when compared with other Enterobacteriaceae. These are:

1 The ability to survive at relatively high osmotic pressure.
2 To multiply at relatively low pH values.
3 To be relatively more resistant to malachite green.
4 To have relatively less demanding nutritional requirements.

RVS Broth is based on the revised formulation described by van Schothorst et al[2], and is recommended as the selective enrichment medium for the isolation of salmonellae from food and environmental specimens. It can also be used to isolate salmonellae from human faeces without the need for pre-enrichment.

RVS Broth is a modification of the Rappaport Vassiliadis (R10) Enrichment Broth described earlier by van Schothorst and Renauld[3]. The modifications to their earlier formula are:

1 The addition of di-potassium hydrogen phosphate to buffer the medium so that the pH is maintained during storage of the prepared broth.
2 Clarifying the optimum concentration of magnesium chloride $6H_2O$.

The two modifications are said to enhance the reliability of the enrichment broth[1]. Peterz et al.[4] have also highlighted the importance of the concentration of magnesium chloride in the final medium.

Technique
Food and environmental specimens.

1 Prepare Buffered Peptone Water (Oxoid CM509) as instructed on the label in volumes of 225ml.
2 Prepare RVS Broth CM866 as instructed.
3 Add 25g or 25ml of the test sample to 225ml of Buffered Peptone Water and incubate at 37°C for 16–20 hours. Transfer 0.1ml of the pre-enrichment peptone water culture to 10ml of RVS Broth and incubate at 42° ±1.0°C for 24 hours.
4 Sub-culture the enrichment broth by streaking onto plates of MLCB Agar CM783 and Brilliant Green Agar (Modified) CM329. Incubate at 35°C for 18–24 hours. Colonies suspected as salmonellae should be confirmed by biochemical or serological methods.

Faecal specimens – no pre-enrichment needed.
Add one or two 3mm loopfuls of liquid faeces (or an emulsion of faeces in saline) to 10ml of RVS Broth CM866 pre-warmed to 42°C. Incubate at 42°C ±1.0°C for 24 hours, and then streak onto selective agars of choice.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Salmonella typhimurium ATCC®14028

Negative control:
Escherichia coli ATCC®25922

Precautions
RVS Broth should not be used if *Salm. typhi* is suspected.

In order to achieve optimum recovery it is recommended that the enrichment broth is incubated at 42°C ± 1.0°C.

References
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MODIFIED SEMI-SOLID RAPPAPORT VASSILIADIS (MSRV) MEDIUM BASE

Code: CM910

A semi-solid medium for the detection of motile Salmonella spp. from food, faeces and environmental samples.

Formula	gm/litre
Tryptose	4.59
Casein hydrolysate	4.59
Sodium chloride	7.34
Potassium dihydrogen phosphate	1.47
Magnesium chloride (anhydrous)	10.93
Malachite green oxalate	0.037
Agar	2.7
pH 5.2 ± 0.2	

MSRV SELECTIVE SUPPLEMENT

Code: SR161

Vial contents (each vial is sufficient to supplement 500ml of MSRV Medium Base)
Novobiocin 10.mg equivalent to 20mg/litre

Directions
Suspend 15.8g of MSRV Medium Base in 500ml of distilled water. Bring to the boil with frequent agitation. DO NOT AUTOCLAVE. Cool to 50°C and aseptically add the contents of 1 vial of MSRV Selective Supplement reconstituted with 2ml of sterile distilled water. Mix well and pour into sterile petri dishes. Do not invert the dishes. Air dry at room temperature for at least one hour. (Plates may be air-dried overnight prior to storage at 2°C to 8°C.) Do not invert the dishes.

Descriptions
Modified Semi-Solid Rappaport Vassiliadis (MSRV) Medium is based on the formulation described by De Smedt et al[1] which has been shown to detect more salmonella-positive samples than the traditional enrichment procedures[1,2]. Further collaborative studies have confirmed these findings[3,4].

Motility enrichment on MSRV Medium has been designed as a simple, sensitive method for the isolation of salmonellae to migrate through the selective medium ahead of competing motile organisms, thus producing opaque haloes of growth.

Further tests can be carried out directly from the migrated culture, the inoculum being taken from the edge of the growth. The Oxoid Salmonella Latex Test (FT203) is recommended for serological confirmation of *Salmonella* species.

MSRV Medium has been employed in combination with direct culture and selenite F broth enrichment for isolation of *Salmonella* spp. from faeces. Subsequent plating to XLD and MLCB Agars resulted in a recovery rate of 98.9% of *Salmonella* spp. in 1544 faeces specimens[5].

The medium is not suitable for the detection of non-motile strains of Salmonella (incidence 0.1%)[6].

(Figures obtained from records of the Department of Enteric Pathogens, Central Public Health Laboratory Colindale, London. Dr. B. Rowe, Personal Communication, 1988).

Technique
1 Inoculate three drops (Ca. 0.1ml) of the pre-enrichment culture (after incubation for 16–20 hours) or a 1/10 suspension of faecal specimen in separate spots on the surface of the MSRV Medium plates.
2 Incubate the plates in an upright position at 41°C–42°C for up to 24 hours. (Care should be taken not to exceed 24 hours.)

Note
When examining faeces the duration of incubation has been shown to be critical[5]. If plates are examined after 18 hours *Salmonella* spp. may be missed because haloes have not developed. Reincubate plates that have been examined at 18 hours for a further 6 hours but do not exceed a total of 24 hours incubation time.

3 Examine the plates for motile bacteria which will be shown by a halo of growth originating from the inoculation spot.
4 Sub-cultures can be taken from the outside edge of the halo to confirm purity and for further biochemical and serological tests.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the selective supplement in the dark at 2–8°C and use before the expiry date on the label.

The prepared medium may be stored for up to 2 weeks at 2–8°C in the dark.

Quality Control
Positive control:
Salmonella typhimurium ATCC® 14028 – Straw colonies at the site of inoculation surrounded by halo of growth.
Salmonella enteriditis ATCC® 13076 – Straw colonies at the site of inoculation surrounded by halo of growth.

Negative control:
Citrobacter fruendii ATCC® 8090 – Restricted or no growth.

Precautions
The basal medium is very hygroscopic and containers must be stored tightly closed.

When handling the powder a face mask and gloves must be worn.

References

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REINFORCED CLOSTRIDIAL AGAR (RCM AGAR)

Code: CM151

A solid medium for the cultivation and enumeration of anaerobes, especially Clostridium species.

Formula	gm/litre
Yeast extract	3.0
'Lab-Lemco' powder	10.0
Peptone	10.0
Glucose	5.0
Soluble starch	1.0
Sodium chloride	5.0
Sodium acetate	3.0
Cysteine hydrochloride	0.5
Agar	15.0
pH 6.8 $\pm$ 0.2	

Directions
Suspend 52.5g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Reinforced Clostridial Agar is a solid version of Oxoid Reinforced Clostridial Medium CM149, suitable for the cultivation and enumeration of clostridia and other anaerobes, lactobacilli, and many other species of bacteria. Reinforced Clostridial Agar does not differ significantly in performance from the pork-starch-pea agar of Anderson[1] for the count of anaerobes. It is employed for the estimation of clostridia in food – see below (also Barnes et al.[2] and Angelotti[3]). Attenborough and Scarr[4] employed RCM Agar, in conjunction with Membrane Filters, for the count of *Clostridium thermosaccharolyticum* in sugar.

Reinforced Clostridial Agar is also frequently employed for the investigation of intestinal flora: Perry et al.[5] for investigation of bovine rumen streptococci; Williams Smith & Crabb[6] used the Oxoid medium with added sodium chloride or blood for counts on human or animal faeces; Barnes & Goldberg[7] employed the Oxoid medium with added chlortetracycline hydrochloride or sodium azide and ethyl violet, for the examination of poultry faeces; the Oxoid medium was also used by Goldberg et al.[8] for the examination of poultry faecal samples. Williams Smith[9] employed Oxoid Reinforced Clostridial Agar with added blood for the 'total' and *Lactobacillus* count of human and animal faeces; with added blood and neomycin for determination of *Bacteroides*, anaerobic Gram-negative cocci, 'total' streptococci, sporeformers, yeasts, and 'actino' types.

Sneath[10] used Oxoid RCM Agar and other media for the anaerobic count of micro-organisms from soil samples up to more than 300 years old. Gregory et al.[11] also employed the Oxoid medium for the estimation of anaerobes in moulding hay.

Technique
Barnes and Ingram[12] and Ingram and Barnes[13] described the use of RCM Agar for the total viable count of clostridia employing their black glass rod technique. In this method the diluted sample is added to plugged test tubes containing about 9ml of RCM Agar held at 48°C. The tubes are quickly rotated to mix the contents, and a sterile black glass rod inserted into each tube before the agar sets. They are then sealed with about 1.5cm of RCM Agar containing 1/20,000 methylene blue. Growth is very rapid in this medium and it is necessary to count the colonies before gas production disrupts the agar. The authors suggest that the tubes should be incubated overnight at 25°C and then at 35°C for several hours. The colonies are clearly visible against the black background.

Oxoid RCM Agar may also be used for enumerating anaerobes using the Miller-Prickett technique (Miller et al.[14]). The Miller-Prickett tube is a flattened test tube 15 x 2.5 x 1.3 cm

Mossel et al.[15], although working with other media, suggested the following procedure which may be used with RCM Agar:

1 Transfer, in triplicate, 1ml of serial decimal dilutions of the food under investigation into sterilised, plugged Miller-Prickett tubes. Cool the freshly prepared medium to approximately 50°C and, without shaking, add about 15ml to each tube.
2 Seal immediately with melted sterile paraffin, and allow to set in a water bath at about 15°C.
3 Incubate for 1 to 10 days at a temperature between 30°C and 55°C, depending on the type of clostridia expected.
4 Run at least one blank to detect contamination occurring during the procedure.
5 Count colonies.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Clostridium perfringens ATCC® 13124

Negative control:
Uninoculated medium

Precautions
Further identification tests must be carried out on organisms isolated from this medium.

References

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REINFORCED CLOSTRIDIAL MEDIUM (RCM)

Code: CM149

A semi-solid medium for the enumeration and cultivation of clostridia and other anaerobes occurring in food and pathological specimens. It is the basal medium for Differential Reinforced Clostridial Medium.

Formula	gm/litre
Yeast extract	3.0
'Lab-Lemco' powder	10.0
Peptone	10.0
Soluble starch	1.0
Glucose	5.0
Cysteine hydrochloride	0.5
Sodium chloride	5.0
Sodium acetate	3.0
Agar	0.5
pH 6.8 $\pm$ 0.2	

Directions
Suspend 38g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A semi-solid medium for the enumeration and cultivation of anaerobes. Recommended for the isolation and cultivation of anaerobic organisms occurring in a variety of habitats, including food and pathological specimens.

Reinforced Clostridial Medium (RCM) was designed by Hirsch & Grinstead[1] for the cultivation and enumeration of clostridia. They showed that the medium was more fertile and enabled growth to be initiated from small inocula more readily than five other media tested. In a further comparison, the highest viable count obtainable was the criterion used, and again, RCM proved superior. Compared with the spleen infusion medium of Mundt & Jones[2], RCM gave somewhat higher counts (Gibbs & Hirsch[3]).

Reinforced Clostridial Medium can be made differential for sulphite-reducing clostridia by the addition of sodium sulphite and ferric citrate[4]. Differential Reinforced Clostridial Medium is recommended for detection of sulphite-reducing clostridia and *Cl. perfringens* in drinking water[5].

Preparation of Differential Reinforced Clostridial Medium
Make separate solutions of 4% sodium sulphite (anhydrous) and 7% ferric citrate in distilled water. Heat the ferric citrate solution to dissolve. Sterilise both solutions by filtration. The solutions may be stored at 4°C for up to 14 days.

On the day of use mix equal volumes of the two solutions. Add 0.5ml of the mixture to each 25ml volume of single-strength freshly steamed and cooled Reinforced Clostridial Medium. To each 10ml and 50ml volume of double-strength medium add 0.4ml and 2ml respectively of the mixed solutions.

All cultures showing blackening must be sub-cultured for confirmatory tests.

Weenk, Fitzmaurice and Mossel[6] modified Differential Reinforced Clostridial Medium by increasing the iron content to 1 gram/litre of ferric ammonium citrate and accurately adjusting the sulphite concentration to 0.05% disodium sulphite heptahydrate. The time required for sulphite-reducing clostridium colonies to blacken was significantly shorter than that when using iron sulphite agar. The modified medium to a great extent suppressed the formation of black colonies by hydrogen sulphide-positive *Bacillus* spp. Resistance to metronidazole and growth on aerobically incubated tryptone soya agar are reliable criteria for recognising *Bacillus* spp. colonies that develop.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Clostridium perfringens ATCC® 13124

Negative control:
Uninoculated medium

Precautions
Further identification tests must be carried out on organisms isolated from this medium.

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ROGOSA AGAR

Code: CM627

A medium for the selective isolation and enumeration of lactobacilli.

Formula	gm/litre
Tryptone	10.0
Yeast extract	5.0
Glucose	20.0
Sorbitan mono-oleate 'Tween 80'	1.0ml
Potassium dihydrogen phosphate	6.0
Ammonium citrate	2.0
Sodium acetate, anhydrous	17.0
Magnesium sulphate	0.575
Manganese sulphate	0.12
Ferrous sulphate	0.034
Agar	20.0
pH 5.4 $\pm$ 0.2	

Directions
Suspend 82 grams in 1 litre of distilled water and bring to the boil to dissolve completely. Add 1.32ml glacial acetic acid and mix thoroughly. Heat to 90–100°C for 2–3 minutes with frequent agitation. Distribute into sterile tubes, petri dishes or bottles. DO NOT AUTOCLAVE.

Description
Rogosa Agar, a modification of the medium described by Rogosa et al.[1], is a selective medium for the isolation and enumeration of lactobacilli. The medium has given excellent results when used in quantitative and qualitative studies of lactobacilli in faeces, saliva and mouth rinses, and in dairy products. It is an effective, selective medium for lactobacilli but the high acetate concentration and low pH suppresses many strains of other lactic acid bacteria.

If the pH of the medium is adjusted to 6.2 without adding acetic acid then the selectivity of the medium is altered to include the whole group of lactic acid bacteria[2,3].

Technique
For the isolation of lactobacilli, Sharpe[4] recommends that Rogosa Agar plates should be incubated for 3 days at 35°C or for 5 days at 30°C. It is preferable to incubate in an atmosphere containing 95% of hydrogen and 5% of carbon dioxide, this prevents evaporation, provides the micro-aerophilic conditions favoured by lactobacilli, and the carbon dioxide has a stimulating effect on their growth. If a suitable container is not available, overlay the inoculated plate with a second layer of Rogosa Agar, before incubation.

Thermophilic lactic acid bacteria are incubated at 42°C for 48 hours and suspected psychrotrophic organisms can be incubated at 30°C for 2 days and at 22°C for a further day. Leuconostocs from meat are incubated at 25°C for 3 days.

After incubation all well grown colonies may be considered as lactic acid bacteria although enterococci and pediococci show a reduced growth rate. Some leuconostocs from meat show slime production at 25°C.

Colony characteristics
Small greyish-white, flat or raised, smooth, rough or intermediate.

Size
Lactobacilli and other lactic acid bacteria 0.5–2.5mm diameter; enterococci 0.5–1.0mm diameter; non-lactic acid bacteria >2.5mm after prolonged incubation at room temperature.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Lactobacillus gasseri ATCC®19992

Negative control:
Staphylococcus aureus ATCC®25923

Precautions
L. carnis does not grow on this medium.

All colonies must be tested by Gram stain and catalase test before carrying out further identification tests.

References

1 Rogosa M., Mitchell J. A. and Wiseman R. F. (1951) *J. Bact. 62. 132–133.*
2 Reuter G. (1985) *Int. J. Food Microbiol. 2. 55–68.*
3 ISO/TC34/SC6/WG15. (1984) *Enumeration of Lactobacteriaceae in meat and meat products.*
4 Sharpe M. Elizabeth (1960) *Lab. Practice 9. 223-227.*

ROSE-BENGAL CHLORAMPHENICOL AGAR

Code: CM549

For the selective enumeration of moulds and yeasts from foods.

Formula	gm/litre
Mycological peptone	5.0
Glucose	10.0
Dipotassium phosphate	1.0
Magnesium sulphate	0.5
Rose-Bengal	0.05
Agar	15.5
pH 7.2 0.2	

CHLORAMPHENICOL SELECTIVE SUPPLEMENT

Code: SR78

Vial contents (each vial is sufficient for 500ml of medium)

Chloramphenicol	50mg

Directions
Suspend 16.0g in 500ml of distilled water and bring to the boil to dissolve completely. Add the contents of one vial of Chloramphenicol Selective Supplement SR78 (reconstituted as directed) and mix gently. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C, mix gently and pour into petri dishes.

Description
Rose-Bengal Chloramphenicol Agar is a selective medium for the enumeration of yeasts and moulds from a wide variety of foodstuffs. The medium has a neutral pH and chloramphenicol is used as a selective agent to suppress the growth of bacteria. Several investigators have noted advantages in the use of media at neutral pH containing antibiotics[1,2]. Rose-Bengal is taken up by mould and yeast colonies thereby assisting enumeration of small colonies[3]. Rose-Bengal also controls the size and height of mould colonies, such as *Neurospora* and *Rhizopus* spp. Over-growth of slow growing strains by more luxuriant species is thus prevented and plate counting is assisted.

The choice of a suitable medium for enumeration of yeasts and moulds is greatly dependent on the nature of the foodstuffs under investigation and the organisms that occur on them[4]. Rose-Bengal Chloramphenicol Agar is recommended for fresh proteinaceous foods whose associated flora consists mainly of Gram negative rod-shaped bacteria although it should be noted that chloramphenicol alone may not be sufficient to inhibit the bacterial background. Because of the stability of chloramphenicol, Rose-Bengal Chloramphenicol Agar is also suitable when higher and prolonged incubation temperatures around 35°C are required.

Technique
Add 1ml aliquots of a suitable series of decimal dilutions to empty 9cm petri dishes. Two dishes are used for each dilution. Then add to each dish approximately 15ml of medium cooled to 50°C. Mix gently, turning the plates three times clockwise and three times counter clockwise.

Allow the medium to gel then turn the petri dishes upside down and incubate them for 5 days at 22°C $\pm 2°$.

Inspect the dishes and count the colonies on those that contain an estimated 50–100 colonies.

Calculate the number of yeasts or moulds per 1g or 1ml by multiplying the number of colonies by the dilution factor.

Consult the appropiate references for further information[5,6,7].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C away from light.

Note
ROSE-BENGAL PHOTO-OXIDISES TO FORM TOXIC COMPOUNDS. STORE PLATES OF THE MEDIUM IN THE DARK AND AVOID EXPOSURE TO LIGHT[8].

Quality Control
Positive control:
Saccharomyces cerevisiae ATCC® 9763
Mucor racemosus ATCC® 42647
Negative control:
Escherichia coli ATCC® 25922
Enterococcus faecalis ATCC® 29212

Precautions
It is essential to store plates of media containing Rose-Bengal in the dark to prevent toxic photo-oxidation of the dye. See above.

Identify moulds and yeasts by morphological appearance and microscopic examination. Colonies of bacteria and yeasts can be confused.

References

1 Mossel D. A. A., Visser M. and Mengerink W. H. J. (1962) *Lab. Pract. 11. 109–112.*
2 Koburger J. A. (1968) *Bact. Proc. 13. A73.*
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5 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.*
6 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.*
7 American Public Health Association (1981) *Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington DC.*
8 Kramer C. L. and Pady S. M. (1961) *Kansas Academy of Science Vol. 64 No. 2 1961. Inhibition of growth of Fungi on Rose-Bengal media by light.*

SABOURAUD DEXTROSE AGAR

Code: CM41

An acid pH medium for the isolation of dermatophytes, other fungi and yeasts.

Formula	gm/litre
Mycological peptone	10.0
Glucose	40.0
Agar	15.0
pH 5.6 ± 0.2	

Directions
Add 65g to 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
This modification of Sabouraud agar (Carlier[1]) is suitable for the cultivation and differentiation of fungi.

Carlier showed that the medium gives reliable results with *Microsporum audouini*, *M. canis*, *Trichophyton mentagrophytes*, *T. flavum*, *T. rubrum* and *Candida albicans*. Sabouraud Dextrose Agar may be used in place of the Standard American medium of Hodges[2]. The fungi maintain their typical cultural appearance and thus may be readily identified according to the standard macroscopic characters described by Sabouraud[3].

The medium is often used with antibiotics for the isolation of pathogenic fungi from material containing large numbers of other fungi or bacteria.

Georg et al.[4] aseptically added 0.5 gram cycloheximide, 20,000 units penicillin and 40,000 units streptomycin to each litre of autoclaved, cooled medium. *Cryptococcus neoformans*, *Aspergillus fumigatus* and *Allescheria boydii* are sensitive to cycloheximide; *Actinomyces bovis* and *Nocardia asteroides* are sensitive to penicillin and streptomycin.

Alternatively, one may add 0.4 gram chloramphenicol and 0.05 gram cycloheximide to each litre of reconstituted medium before autoclaving (Ajello[5]). The same micro-organisms are sensitive to this new combination – see Dermasel Selective Supplement SR75.

Williams Smith & Jones[6] employed Oxoid Sabouraud Dextrose Agar, containing 20,000 units penicillin and 0.04 gram neomycin per litre, for the count of yeasts in the alimentary tract of the pig.

Hantschke[7] used colistin, novobiocin and cycloheximide to isolate *Candida albicans*. Dolan[8] used gentamicin, chloramphenicol and cycloheximide for the selective isolation of pathogenic fungi.

Oxoid Sabouraud Dextrose Agar may also be used as the basis of a Pagano-Levin medium[9] for the isolation of *Candida albicans*. 0.1 gram of triphenyltetrazolium chloride (as a filter sterilised solution) is added to each litre of autoclaved molten medium cooled to 55°C. The medium is usually made inhibitory to most non-pathogenic fungi and bacteria by the addition of antibiotics as above. After incubation for 3 days at 25°C, *Candida albicans* colonies are unpigmented or pale pink whilst other *Candida* species and other fungi form deeper pink or red colonies. The test is adequate for screening purposes but other diagnostic criteria should also be utilised for the identification of *Candida albicans*[10,11,12,13].

Technique
1 Inoculate each specimen in duplicate.
2 Incubate one set of media aerobically at 22–25°C and the other set at 35°C for 5–30 days. Loosen the caps of tubes and ensure adequate moisture for the plates to compensate for loss of water vapour. DO NOT SEAL THE PLATES.
3 Examine every 2–4 days.
4 Describe each specific type of colony morphology and sub-culture to appropriate media for further identification tests.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Trichophyton rubrum ATCC® 28191
Candida albicans ATCC® 10231

Negative control:
(with antibiotics)
Staphylococcus aureus ATCC® 25923
Escherichia coli ATCC® 25922

Precautions
Some of the pathogenic fungi may produce infective spores which are are easily dispersed into the laboratory. Such organisms should be examined only within a protective cabinet.

The combination of cycloheximide and chloramphenicol inhibits many pathogenic fungi[4]. However, the mycelial phase of *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Sporothrix schoenckii* and *Blastomyces dermatitidis* is not inhibited by these antibiotics when incubated at 25–30°C[14].

Note the precautions in handling cycloheximide described in HAZARDS page 2–7.

References

1 Carlier Gwendoline I. M. (1948) *Brit. J. Derm. Syph. 60. 61–63.*
2 Hodges R. S. (1928) *Arch. Derm. Syph., New York, 18. 852.*
3 Sabouraud R. (1910) *'Les Teignes', Masson, Paris.*
4 Georg Lucille K., Ajello L. and Papageorge Calomira (1954) *J. Lab. Clin. Med. 44. 422–428.*
5 Ajello Libero (1957) *J. Chron. Dis. 5. 545–551.*
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9 Pagano J., Levin J. G. and Trejo W. (1957–58) *Antibiotics Annual 1957–58, 137–143.*
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12 Sinski J. T. (1960) *J. Invest Dermat. 35. 131–133.*
13 Ridley M. F. (1960) *Australian J. Dermat. 5. 209–213.*
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SABOURAUD LIQUID MEDIUM

Code: CM147

A liquid medium recommended for sterility testing and for the determination of the fungistatic activity of pharmaceutical products.

Formula	gm/litre
Pancreatic digest of casein	5.0
Peptic digest of fresh meat	5.0
Glucose	20.0
pH 5.7 ± 0.2	

Directions
Dissolve 30g in 1 litre of distilled water. Mix well, distribute into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
Sabouraud Liquid Medium is a mycological sterility test medium conforming to the medium described in the USP[1] and FDA Bacteriological Analytical Manual[2] for the determination of the fungistatic activity of pharmaceutical and cosmetic products in order to avoid false sterility tests. The medium may also be used for the cultivation of moulds, yeasts, and acidophilic bacteria.

In clinical microbiology, the use of Sabouraud Liquid Medium has been shown to increase the isolation rate of *Candida albicans* in blood culture[3].

Technique
The USP recommends that the fungistatic activity of pharmaceutical products be determined as follows:

1 ***Test Culture***
A 1 in 1,000 dilution of a 24 to 28 hour culture of *Candida albicans* in Sabouraud Liquid Medium and inoculate with the Test Culture.

2 ***Tests***
Add specified amounts of the product to be tested to volumes of Sabouraud Liquid Medium and inoculate with the Test Culture.

3 ***Controls***
Inoculate tubes of Sabouraud Liquid Medium only, with the Test Culture.

4 Incubate at 22°C to 25°C for at least 10 days.

5 If growth in the test series is comparable to that in the control tubes, then the product is not fungistatic – therefore use the amount of product and medium specified for all routine sterility tests on the product.

If the product is fungistatic when tested as above, add a suitable sterile inactivating reagent, or, use a larger ratio of medium to product in order to determine the ratio of product to medium in which growth of the test organism is not affected.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium below 25°C.

Quality Control
Positive control:
Candida albicans ATCC® 102131
Aspergillus niger ATCC® 9642

Negative control:
Uninoculated medium

References
1 Pharmacopoeia of the United States: 1995 *Sterility Testing.*
2 Food and Drug Administration (1992) *Bacteriological Analytical Manual 7th Ed. F.D.A. Washington D.C.*
3 Reeder J.C., Ganguli L.A., Drucker D.B., Keaney M.G.L. and Gibbs A.C.C. (1989) *Microbios. 60. 71–77.*

SABOURAUD MALTOSE AGAR

Code: CM41a

An acid medium for the isolation of dermatophytes, other fungi and yeasts.

Formula	gm/litre
Mycological peptone	10.0
Maltose	40.0
Agar	15.0
pH 5.6 ± 0.2	

Directions
Suspend 65g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
This medium differs from Sabouraud Dextrose Agar, only in the carbohydrate incorporated. Sabouraud Maltose Agar may be used, with or without antibiotics, where a maltose medium is preferred.

Sabouraud Maltose Agar may be modified to form a selective indicator medium for the isolation of *Candida albicans* by the addition of Tergitol-7, bromocresol purple, potassium tellurite and triphenyltetrazolium chloride (Chapman[1]).

The storage conditions, quality control tests and precautions to be observed are exactly those described under Sabouraud Dextrose Agar CM41.

Reference
1 Chapman G. H. (1952) *Trans. New York Acad. Sci., Series II 14(6). 254.*

SALT MEAT BROTH

Code: CM94 (Tablets)

An enrichment broth for halophilic organisms, especially staphylococci.

Formula	gm/litre
Peptone	10.0
'Lab-Lemco' powder	10.0
Neutral heart muscle	30.0
Sodium chloride	100.0
pH 7.6 ± 0.2	

Directions
Add 2 tablets to 10ml of distilled water in a 5/8 in. diameter test tube and soak for 5 minutes. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Salt Meat Broth is an enrichment medium for the isolation of staphylococci from grossly contaminated specimens such as faeces, particularly during the investigation of staphylococcal food poisoning. A salt meat medium will detect small numbers of staphylococci when mixed with large numbers of other bacteria[1,2].

The medium is also an excellent substrate for the cultivation of some of the halophilic micrococci associated with hides and raw salt supplies. It should be noted that staphylococci growing on this medium cannot be directly tested for coagulase production – they should first be sub-cultured on a medium which contains less salt. Blood Agar Base CM55 is recommended for this purpose.

Technique
For the isolation of *Staphylococcus aureus* from samples of food, emulsify the specimen in Peptone Water CM9, and inoculate a tube of Salt Meat Broth. After 24 to 48 hours' incubation at 35°C, discrete colonies may be obtained by plating out a small portion of the liquid culture on Mannitol Salt Agar CM85 or Staphylococcus Medium No.110 CM145.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium below 25°C.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923

Negative control:
Escherichia coli ATCC® 25922

References

1 Maitland H. B. and Martyn G. (1948) *J. Path. Bact. 60. 553–557.*
2 Fairbrother R. W. and Southall J. E. (1950) *Mon. Bull. Min. Hlth Pub. Hlth Serv. 9. 170–172.*

SCHAEDLER ANAEROBE AGAR

Code: CM437

A medium free from thioglycollate for the growth of aerobic and anaerobic organisms.

Formula	gm/litre
Tryptone Soya Broth (Oxoid CM129)	10.0
Special peptone	5.0
Yeast extract	5.0
Glucose	5.0
Cysteine HCl	0.4
Haemin	0.01
Tris Buffer	0.75
Agar	13.5
pH 7.6 ± 0.2	

Directions
Suspend 40g in 1 litre of distilled water and boil to dissolve the medium completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring.

Description
Schaedler, Dubos and Costello[1] formulated this medium for the isolation of aerobic and anaerobic micro-organisms from the gastro-intestinal tract of mice. Mata, Carrillo and Villatoro[2] modified the formula in their studies on anaerobic human faecal microflora. The modified formula has been used in Oxoid Schaedler Anaerobe Agar/Broth and the medium can be used to create selective conditions under which the required, delicate and more nutritionally exacting micro-organisms of the intestinal tract would develop, despite the presence of antagonistic organisms. Normally such fastidious micro-organisms would be swamped by the growth of enterococci, coliform bacilli and other Gram-negative bacilli.

In both studies[1,2] the use of the base medium with selective agents (shown overleaf) for the isolation and enumeration of *Lactobacillus*, *Streptococcus*, *Clostridium*, *Bacteroides* and *Flavobacterium* species from faecal samples and various organs of the digestive tract.

Although thioglycollate is widely used in anaerobic media, to lower the redox potential in order to favour growth of anaerobic organisms, some workers have reported it to be inhibitory to some anaerobes[3,4].

Schaedler Anaerobe Agar CM437 contains cysteine hydrochloride and glucose, as reducing substances, with the advantage that cysteine inhibits the growth of *Escherichia coli.* Kari, Nagy, Kovacs & Hernadi[5] have reported the inhibitory effect of cysteine on several enzymatic reactions of *Esch. coli in vitro.*

Schaedler Anaerobe Agar CM437 has been shown to be a suitable alternative to blood agar for the enumeration of clostridia[6] and has been used for the examination of food, waste products and ditch water[7]. These authors showed the necessity for strict anaerobic conditions for the successful recovery of obligate anaerobes when using this medium without the addition of blood.

Investigations at Oxoid have shown that Schaedler Anaerobe Agar CM437 gave similar results in recovery and colonial appearance to Oxoid Blood

Agar Base No.2 when tested with the same organisms.

Technique
The sample suspension is diluted as necessary in order to obtain separated and countable colonies. A calibrated loopful is then spread on the surface of a previously dried Schaedler Anaerobe Agar plate. Conditions of incubation will vary according to the type of culture under test. Pure cultures may grow on the base medium and this is also used for general aerobic and anaerobic counts.

In the enumeration of *Enterococcus faecalis* (facultatively anaerobic) as an indicator organism in dehydrated or frozen foods and water, and for the detection of *Clostridium*, the medium can be used as follows:

Food sample (e.g. pre-cooked frozen food) suspensions are plated out by the surface spread technique and an aerobic viable count may be carried out at 25°C and 35°C. For pre-cooked meat products, an anaerobic viable count and a selective plate examination for *Cl. perfringens* should also be performed.

Addition of Selective Agents
To 1,000ml of base agar, the following selective agents may be added:

1 Medium for anaerobic lactobacilli and anaerobic streptococci.

NaCl	10.0g
Neomycin	0.002g

Incubate anaerobically at 35°C.

2 Medium for bacteroides and clostridia.

Placenta powder (Nutritional Biochemicals Corp, Cleveland)	2.0g
Neomycin	0.002g

Incubate anaerobically at 35°C.

3 Medium for flavobacteria.
7ml of 0.5% tyrothricin in ethanol.
Incubate aerobically at 35°C.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Prepared plates may be stored at 2–8°C if suitably protected.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923
Clostridium perfringens ATCC® 13124
Bacteroides fragilis ATCC® 23745

Negative control:
Uninoculated medium.

Precautions
Note the comment on strict anaerobic conditions for obligate anaerobe isolation without blood.

References

1 Schaedler R. W., Dubos R. and Castello R. (1965) *J. Exp. Med. 122. 59–66.*
2 Mata L. J., Carrillo C. and Villatoro E. (1969) *Appl. Microbiol. 17. 596–599.*
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SCHAEDLER ANAEROBE BROTH

Code: CM497

A broth version of Schaedler Anaerobe Agar (CM437) for the general growth of anaerobes, for use in blood cultures and antibiotic MIC studies of these organisms.

Formula	gm/litre
Tryptone Soya Broth (Oxoid CM129)	10.0
Special peptone	5.0
Yeast extract	5.0
Glucose	5.0
Cysteine HCl	0.4
Haemin	0.01
Tris buffer	0.75
pH 7.6 ± 0.2	

Directions
Add 26.5g to 1 litre of distilled water and mix to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Schaedler Anaerobe Broth CM497 is a clear medium which can support the growth of those anaerobic bacteria commonly associated with human and veterinary disease. It is identical to the formula of Schaedler Anaerobe Agar CM437, except that the agar has been omitted.

Used as a fluid medium, under the appropriate atmosphere, Schaedler Anaerobe Broth CM497 showed enhanced growth with a number of demanding anaerobic organisms when compared with seven other commonly used broth media[1].

Schaedler Anaerobe Broth CM497 can also be used to determine antibiotic MIC levels of anaerobic organisms. The extreme variations of growth rates usually prevent the existing linear regression plots of MIC versus zone diameter being used. The use of tube methods overcomes this problem[1].

Fass, Prior and Rotilie[2] described a simple tube method that does not require special atmospheres or special equipment to carry out the test. By adding a 6mm solid glass bead to the tube of broth prior to autoclaving, growth of most organisms could be detected after one day's incubation, by slowly rotating the tube and observing the swirl of organisms. The addition of 0.0001 of w/v resazurin to the medium was used to determine whether oxidation had occurred in stored media. For anaerobic cocci, heat-inactivated horse serum was added to a final concentration of 1% v/v before use[3].

The addition of menadione (0.1g/litre), sodium polyanethol-sulphonate (SPS, 0.3g/litre) and carbon dioxide (3% v/v) to Schaedler Broth enables it to be used as a blood culture medium and for the cultivation of especially fastidious *Bacteroides* species.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store prepared broth in the dark at low ambient temperature <15°C.

Quality Control
Positive control:
Clostridium perfringens ATCC® 13124
Bacteroides fragilis ATCC® 25285
Prevotella loescheii ATCC® 15930 (with menadione addition)

Negative control:
Uninoculated.

Precautions
As with all anaerobic broth media, it is important to avoid chemo-oxidation (overheating) and photo-oxidation (storage in the light) because such oxidative effects will cause inhibition of growth.

References

1 Stalons D. R., Thornsberry C. and Dowel V. R. (1974) *Appl. Microbiol. 27. 1098–1104.*
2 Fass R. J., Prior R. B. and Rotilie C. A. (1975) *Antimicrob. Agents Chemother. 8. 444–452.*
3 Rotilie C. A., Fass R. J., Prior R. B. and Perkins R. L. (1975) *Antimicrob. Agents Chemother. 7. 311–315.*

SELENITE BROTH BASE (LACTOSE)

Code: CM395

An enriched medium for the isolation of Salmonella from faeces and food products.

Formula	gm/litre
Peptone	5.0
Lactose	4.0
Sodium phosphate	10.0
pH 7.1 ± 0.2	

Directions
Dissolve 4 grams of sodium biselenite L121 in 1 litre of distilled water and then add 19 grams of CM395. Warm to dissolve, mix well and fill out into containers to a depth of 5cm. Sterilise in a boiling water bath, or in free flowing steam, for 10 minutes. DO NOT AUTOCLAVE.

To minimise any possible risk of teratogenicity to laboratory workers, the sodium biselenite must be added as a solution to this medium.

Robertson[7] reported miscarriages and possible teratogenic effects on pregnant laboratory assistants which may have been caused by ingested sodium biselenite. Oxoid therefore removed this substance from the powdered medium.

Although no further reports have been received sodium biselenite is now considered to be very toxic and should be handled with great care.

SODIUM BISELENITE (SODIUM HYDROGEN SELENITE)

Code: L121

Directions
Dissolve 4g in 1 litre of distilled water and use this solution to reconstitute the base medium or tablets.

Toxic by inhalation and if swallowed. Danger of cumulative effects.

Description
Klett[1] first demonstrated the selective inhibitory effects of selenite and Guth[2] used it to isolate *Salmonella typhi*. It was twenty years later before Leifson[3] fully investigated selenite and promoted wide use of the medium.

Selenium toxicity to certain micro-organisms is not fully understood but it is suggested that it reacts with sulphur and sulphydral groups in critical cell components[4,5].

Proteus and *Pseudomonas* species appear to be resistant to its effects[4]. Lactose is added as a fermentable carbohydrate to prevent a rise in pH value during incubation because any increase in pH will reduce the selective activity of selenite. The fact that *Proteus* and *Pseudomonas* species do not ferment lactose may explain why they escape inhibition.

There have been many modifications and alterations to the original medium described by Leifson, including mannitol to replace lactose (Mannitol Selenite Broth CM399), addition of cystine (Selenite Cystine Broth CM699), brilliant green, sodium taurocholate, sulphapyridine and streptomycin. The performance of these modifications has been investigated but with no overall agreement[6].

Technique
For routine purposes Selenite Broth cultures should be incubated at 35°C for 18 to 24 hours and then sub-cultured on any combination of greater and lesser inhibitory selective agars for Enterobacteriaceae. The development of *Escherichia coli* and *Proteus* species is not indefinitely retarded in selenite media. Where the initial proportion of these organisms is high, it is often advantageous to sub-culture on to the solid media after 6 hours as well as after 18 hours.

If a high proportion of debris is present, in the sample of material being examined, the selective powers of the selenite may be nullified. This is well established in the examination of faeces and egg powder. It is common practice to emulsify the specimen in sterile saline, allow the gross particles to settle, and inoculate the medium with the supernatant. An alternative method is as follows: Add 2 to 3 grams of solid specimen to 15ml of saline in a wide-necked 1oz. bottle, emulsify, separate the debris by slowly pressing a plug of cotton-wool down through the suspension. Withdraw approximately 1ml of the supernatant and inoculate 10ml of Selenite Broth.

Harvey & Scott Thomson[2] showed that incubation of the selenite broth at 43°C facilitated the isolation of *Salmonella paratyphi B* from faeces. They recommended the use of this principle for the examination of sewage and river water containing large numbers of other bacteria that preferred a lower temperature for growth. The authors also suggested that the procedure was of value for all salmonellae except *Salmonella typhi*. For urines, the broth should be made double strength and inoculated with its own volume of the specimen.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922

Sub-culture to MacConkey Agar.

Precautions
Discard the prepared medium if large amounts of reduced selenite can be seen as a red precipitate in the bottom of the bottles.

Do not incubate longer than 24 hours because the inhibitory effect of selenite is reduced after 6–12 hours incubation[10].

Take sub-cultures of broth from the upper third of the broth column, which should be at least 5cm in depth.

References

1 Klett A. (1900) *Zeitsch. für Hyg. und Infekt. 33. 137–160.*
2 Guth F. (1916) *Zbl. Bakt. I. Orig. 77. 487–496.*
3 Leifson E. (1936) *Amer. J. Hyg. 24. 423–432.*
4 Weiss K. F., Ayres J. C. and Kraft A. A. (1965) *J. Bact. 90. 857–862.*
5 Rose M. J., Enriki N. K. and Alford J. A. (1971) *J. Food Sci. 36. 590–593.*
6 Fagerberg D. J. and Avens J. S. (1976) *J. Milk Food Technol. 39. 628–646.*
7 Robertson D. S. F. (1970) *Lancet i. 518–519.*
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9 Harvey R. W. S. and Price T. H. (1979) *J. Appl. Bact. 46. 27–56.*
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MANNITOL SELENITE BROTH BASE

(See Selenite Broth Base)

Code: CM399 – Sodium Biselenite **Code:** L121

A modification of Selenite F Broth especially recommended for the enrichment of salmonellae.

Formula	gm/litre
Bacteriological peptone	5.0
Mannitol	4.0
Sodium phosphate	10.0
pH 7.1 ± 0.2	

Directions
Add 19 grams to 1 litre of distilled water to which 4 grams of sodium biselenite L121 has been added.

Warm to dissolve, mix well and fill out into containers to a depth of 5cm. Sterilise in a boiling water bath, or in free flowing steam, for 10 minutes.

DO NOT AUTOCLAVE.

To minimise any possible risk of teratogenicity to laboratory workers, the sodium biselenite must be added to this medium separately.

Description
This medium is similar to the modification of Leifson[1] enrichment medium described by Hobbs & Allison[2] for the isolation of *Salmonella typhi* and *Salmonella paratyphi B.*

Hobbs & Allison[2] compared two sets of selenite media, one containing lactose and the other mannitol. Of 38 positive stools *S. typhi.* was sub-cultured from both media in 32 instances, from the mannitol selenite alone in 5 instances and from the lactose selenite alone once. Comparisons showed that the mannitol selenite broth was superior to three other liquid media in its selective value for *S. typhi* and that it was as good as tetrathionate for the isolation of *S. paratyphi B.*

Technique
Sub-cultures from this selective, enrichment broth can be made to any combination of greater and lesser inhibitory selective agars for the Enterobacteriaceae.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922

Sub-culture to MacConkey Agar.

Precautions
Observe the precautionary comments made about sodium biselenite in Selenite Broth Base CM395.

Discard the prepared medium if large amounts of reduced selenite can be seen as a red precipitate in the bottom of the bottle.

Do not incubate longer than 24 hours because the inhibitory effect of selenite is reduced after 6–12 hours incubation.

Mannitol fermentation by salmonella helps correct the alkaline pH swing which can occur during incubation.

Take sub-cultures of broth from the upper third of the broth column, which should be at least 5cm in depth.

References

1 Leifson E. (1936) *Am. J. Hyg. 24(2). 423–432.*
2 Hobbs Betty C. and Allison V. D. (1945) *Mon. Bull. Min. Hlth Pub. Hlth Lab. Serv. 4. 12–19.*

SELENITE CYSTINE BROTH BASE

Code: CM699

An enrichment medium for the isolation of salmonellae from faeces and food products.

Formula	gm/litre
Tryptone	5.0
Lactose	4.0
Disodium phosphate	10.0
L-Cystine	0.01
pH 7.0 ± 0.2	

Directions
Dissolve 4g of sodium biselenite L121 in 1 litre of distilled water and then add 19g of Selenite Cystine Broth Base CM699. Warm to dissolve and dispense into containers to a depth of at least 60mm. Sterilise by placing in free flowing steam for 15 minutes. DO NOT AUTOCLAVE.

To minimise any possible risk of teratogenicity to laboratory workers the sodium biselenite is not included in the dry powder but should be prepared separately as a solution to which the Selenite Cystine Broth Base is added.

Description
Selenite Cystine Broth Base CM699 is modified from the formula of Leifson[1] with added cystine[2]. This addition has given favourable results in many studies[3].

The effect of the cystine may be due to its reducing abilities which will lower the toxicity of selenite to micro-organisms and/or the extra organic sulphur provided may have a sparing effect on the critical sulphur components of the bacteria, again reducing the selective effect of the selenite.

Selenite Cystine Broth is used for enrichment culture of salmonellae from faeces, foodstuffs and other materials. The formulation corresponds to that recommended by the AOAC[4] for detection of *Salmonella* in foodstuffs, in particular egg products. It is included among the standard methods media of the American Public Health Association[5,6]. It also complies with the requirements of the United States Pharmacopoeia[7].

Technique
The proportion of sample in the enrichment broth should not exceed 10–20% (1 or 2 grams in 10–15ml). Solid material is added to the normal strength broth. Liquid samples are mixed with double strength medium in the ratio of 1 to 1. Incubate for 12–24 hours at 35–37°C. Some workers have recommended that 43°C be used[8,9].

Sub-culture to any combination of greater and lesser inhibitory, selective agars for the Enterobacteriaceae.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922

Sub-culture to MacConkey Agar.

Precautions
Observe the precautionary comments made about sodium biselenite in Selenite Broth Base CM395.

Discard the prepared medium if large amounts of reduced selenite can be seen as a red precipitate in the bottom of the bottle.

Do not incubate longer than 24 hours because the inhibitory effect of selenite is reduced after 6–12 hours incubation[10].

Take sub-cultures of broth from the upper third of the broth column which should be at least 5cm in depth.

References

1 Leifson E. (1936) *Am. J. Hyg. 24(2) 423–432.*
2 North W. R and Bartram M. T. (1953) *Appl. Microbiol. l. 130–134.*
3 Fricker C. R. (1987) *J. Appl. Bact. 63. 99–116.*
4 Association of Official Analytical Chemists (1978) *Bacteriological Analytic Manual. 5th Edn. AOAC. Washington DC.*
5 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.*
6 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.*
7 United States Pharmacopoeia XXI (1980) *Microbial Test Limits.*
8 Harvey R. W. S. and Scott T. (1953) *Mon. Bull. Min. Hlth & PHLS. 12. 149–150.*
9 Harvey R. W. S. and Price T. H. (1979) *J. Appl. Bact. 46. 27–56.*
10 Chattopadhyay W. and Pilford J. N. (1976) *Med. Lab. Sci. 33. 191–194.*

SENSITEST AGAR

Code: CM409

A medium specially designed to give large, clear zones with all antibiotics, without the addition of lysed or whole blood.

Formula	gm/litre
Hydrolysed casein	11.0
Peptones	3.0
Sodium chloride	3.0
Glucose	2.0
Starch	1.0
Buffer salts	3.3
Nucleoside bases	0.02
Thiamine	0.00002
Agar	8.0
pH 7.4 ± 0.2	

Directions
Suspend 32g in 1 litre of distilled water and bring to the boil to dissolve the agar. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Sensitest Agar CM409 was developed in the Oxoid laboratories as an antimicrobial susceptibility testing medium that did not require the addition of lysed horse blood to overcome sulphonamide and trimethoprim antagonists.

Using hydrolysed casein as the major source of amino-nitrogen in the medium, it was possible to lower the peptone content to the minimum necessary to supply essential peptides and other growth factors. Careful control of the hydrolysis of the peptones ensures that antagonists to critical antibiotics do not arise.

Bell[1] in a monograph on antimicrobial susceptibility testing chose Oxoid Sensitest Agar as the preferred medium, from those tested, on the following criteria:

1 Ability to support the growth of common Gram positive and Gram negative organisms under the conditions of test.
2 Ability to yield reproducible results.
3 Did not require the addition of lysed horse blood when a heavy inoculum method was employed.
4 Ability to demonstrate standard zones of inhibition with reference organisms and antibiotics.

In the final development of the CDS method Bell selected Sensitest Agar because of its superiority in sulphonamide testing, easier reconstitution of the dehydrated powder and stability of the powdered medium on storage.

Whilst the addition of 5% horse blood to the medium is required for demanding strains, e.g. *Strept. pyogenes* and *Strept. pneumoniae*, there is no significant difference in zone sizes from the addition of blood.

The agar used in the medium, has been specially processed to yield a gel that does not impede the diffusion of antimicrobials.

For further details of antimicrobial susceptibility testing see Section 6.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of agar at 2–8°C.

Quality Control
Positive control:
Escherichia coli ATCC® 25922
Pseudomonas aeruginosa ATCC® 27853
Staphylococcus aureus ATCC® 25923
Enterococcus faecalis ATCC® 29212

Negative control:
Uninoculated medium

Precautions
As with other susceptibility testing media, Sensitest Agar should be used for rapidly growing aerobic organisms only. It should not be modified by the addition of carbohydrates or incubated in a CO_2 enriched atmosphere.

If the medium is used for Bell's CDS method then the specified discs and technique must be used. Refer to the monograph cited in the References.

Reference
1 Bell S. M. (1975) *Supplement to Pathology (Journal of the Royal College of Pathology of Australia) Vol.7 No.4. pp. 1–48.*

SIMMONS CITRATE AGAR

Code: CM155

An agar medium for the differentiation of Enterobacteriaceae based on the utilisation of citrate as the sole source of carbon.

Formula	gm/litre
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	0.2
Sodium ammonium phosphate	0.8
Sodium citrate, tribasic	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
Agar	15.0
pH 7.0 ± 0.2	

Directions
Suspend 23g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Simmons Citrate Agar is recommended (Ewing and Edwards[1]) for the differentiation of the family Enterobacteriaceae based on whether or not citrate is utilised as the sole source of carbon.

The medium is virtually a solidified form of Koser citrate medium which, in its original form, suffered from the disadvantage that false appearance of growth occurred when large inocula were employed. The addition of bromothymol blue indicator to the medium was a distinct improvement. See Koser Citrate Medium CM65.

Simmons Citrate Agar complies with the recommendations of the APHA[2].

Technique
The medium may be used either as slopes in test tubes or as a plate medium in petri dishes. In both cases the surface of the medium is lightly inoculated by streaking and, where slopes are used, the butt of medium is inoculated by stabbing. Incubation for 48 hours at 35° is recommended.

Positive growth (i.e. citrate utilisation) produces an alkaline reaction and changes the colour of the medium from green to bright blue, whilst in a negative test (i.e. no citrate utilisation) the colour of the medium remains unchanged.

Escherichia coli including serotypes from epidemic infantile enteritis, as well as *Shigella, Yersinia* and *Edwardsiella* species do not grow on the medium. *Serratia* and the majority of the *Enterobacter, Citrobacter, Klebsiella, Proteus* and *Providence* species, except *Morganella morganii* and *Klebsiella rhinoscleromatis* utilise citrate and produce the characteristic blue coloration[3].

Simmons Citrate Agar may be used to differentiate citrate-positive *Salmonella enteritidis* and members of

Salmonella subgenus II, III and IV from the citrate-negative *Salmonella typhi, Salmonella paratyphi A, Salmonella pullorum* and *Salmonella gallinarum*.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Klebsiella pneumoniae ATCC® 13883

Negative control:
Escherichia coli ATCC® 25922

Precautions
It is important not to carry over any nutrients into the citrate medium because this will result in false positive tests. Dilute the inoculum in saline before inoculating the citrate medium to avoid a carry-over of other carbon sources[4].

References

1 Ewing W. H. and Edwards P. R. (1960) *Bull. Bact. Nomen. and Taxon. 10. 1–12.*
2 American Public Health Association (1981) *Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington DC.*
3 Kauffman F. (1954) *'Enterobacteriaceae' 2nd ed., Munksgaard, Copenhagen.*
4 Matsen J. M. and Sherris J. C. (1969) *Appl. Microbiol. 18. 452–454.*

SIM MEDIUM

Code: CM435

A medium for the differentiation of enteric bacteria on the basis of sulphide production, indole production and motility.

Formula	gm/litre
Tryptone	20.0
Peptone	6.1
Ferrous ammonium sulphate	0.2
Sodium thiosulphate	0.2
Agar	3.5
pH 7.3 ± 0.2	

Directions
Suspend 30g in 1 litre of distilled water and boil to dissolve the medium completely. Dispense into final containers and sterilise by autoclaving for 15 minutes at 121°C.

Description
A motility-indole medium has been found to be helpful in the identification of the Enterobacteriaceae; e.g. in the differentiation of *Klebsiella* from *Enterobacter* and *Serratia* species[1]. For convenience, these two important tests have been combined with sulphide-production in one tube.

The production of hydrogen sulphide is a useful diagnostic test in the identification of enteric bacteria and is helpful in the differentiation between *Salmonella* and *Shigella*. The sulphate-reducing bacteria will produce hydrogen sulphide and further chemical substitution results in ferrous sulphide being formed along the line of inoculation.

The presence of fermentable sugars may suppress the enzyme mechanism which forms hydrogen sulphide, as a result of the acid products formed (Bulmash and Fulton[2]) and therefore sugars are not included in the medium. Oxoid SIM Medium can be used in conjunction with Triple Sugar Iron Agar CM277 to assess the ability of the culture to ferment lactose, sucrose and glucose.

The production of indole from tryptophan is one of the diagnostic tests used in identifying enteric bacteria. For example, unless it is an unusual form, a *Salmonella* culture never produces indole from tryptophan in amounts detectable in usual tests.

Tryptone is incorporated into the medium since it is a tryptophan-rich peptone, and after incubation, indole can be identified by a red dye complex reaction with one of several reagents, e.g. Kovac's Reagent which consists of amyl alcohol, para-dimethylaminobenzaldehyde and concentrated hydrochloric acid[3].

The presence of glucose in the medium is avoided as recommended[4]. False negative reactions have been recorded when fermentation has occurred[5].

The use of only 0.35% agar in the medium results in the production of a semi-solid medium, ideal for the examination of motility. Non-motile organisms will grow along the line of inoculation only, whereas motile species will grow away from it.

SIM Medium CM435 is therefore designed to determine three characteristics: hydrogen sulphide production, indole production and motility.

Technique
The medium should be dispensed in tubes or bottles and when cool, inoculated once with a pure culture, by inserting a straight wire to about one third of the depth of the medium. If papers are used for the detection of indole, then these are wedged between the cotton wool plug or cap, and side of the container.

The inoculated medium is incubated at 35°C for 18 hours or longer, if necessary, and examined for motility, hydrogen sulphide production and finally indole production from tryptophan.

To Test for Indole Production:
1 Add 0.2ml of Kovac's Reagent to the tube and allow to stand for 10 minutes. A dark red colour in the reagent constitutes a positive indole test. No change in the original colour of the reagent constitutes a negative test.

or

2 Suspend a strip of filter paper, soaked in a solution of saturated oxalic acid and dried, over the medium[4]. Indole formed by positive organisms is volatile and causes the test paper to turn pink.

Colonial Appearances
Non-motile organisms grow only along the line of inoculation, whereas motile species show either a diffuse even growth spreading from the inoculum,

turbidity of the whole medium, or more rarely, localised outgrowths which are usually fan-shaped or occasionally nodular.

Hydrogen sulphide production is shown by blackening of the line of inoculation.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control

Organism	*Motility*	*H_2S*	*Indole*
Escherichia coli ATCC® 25922	V	–	+
Proteus vulgaris ATCC® 13315	+	+	+
Shigella sonnei ATCC® 25931	–	–	–

Precautions
To avoid delay in initiating growth always sub-culture from solid media. The reactions given by SIM Medium are not sufficient to speciate organisms. Additional biochemical and serological tests are required for confirmation.

References

1 Blazevic D. J. (1968) *Appl. Microbiol. 16. 668.*
2 Bulmash J. M. and Fulton M. (1964) *J. Bact. 88. 1813.*
3 Harrigan W. F. and McCance M. E. (1966) *'Laboratory Methods in Microbiology' Academic Press. 53.*
4 Wilson G. S. and Miles A. A. (1964) *Topley and Wilson's 'Principles of Bacteriology and Immunity' 5th ed., Arnold, 1. 490.*
5 Giles R. R. (1956) *J. Clin. Path. 9. 368–371.*

SLANETZ AND BARTLEY MEDIUM

Code: CM377

A medium for the detection of enterococci.

Formula	gm/litre
Tryptose	20.0
Yeast extract	5.0
Glucose	2.0
Disodium hydrogen phosphate	4.0
Sodium azide	0.4
Tetrazolium chloride	0.1
Agar	10.0
pH 7.2 ± 0.2	

Directions
Suspend 42 grams in 1 litre of distilled water and bring to the boil to dissolve the agar completely. EXCESSIVE HEATING MUST BE AVOIDED. Dispense into petri dishes and allow to solidify. It should not be remelted. The medium may be used with membrane filters or by spreading dilutions of the sample over the surface of the agar with a glass rod.

Description
Slanetz & Bartley[1] originally devised this medium to detect and enumerate enterococci by the technique of membrane filtration, but it has also proved useful as a direct plating medium[2,3].

The medium is very selective for enterococci and, when it is incubated at elevated temperatures (44–45°C), all red or maroon colonies may be accepted as presumptive enterococci[4,5].

Burkwall and Hartman showed that the addition of 0.5ml of 'Tween 80' and 20ml of a 10% solution of sodium carbonate or bicarbonate to each litre of medium was of value when examining frozen foods for enterococci; the original article should be consulted for procedural details[2].

Technique
The Department of Health[6] in their 'Report 71' recommend the use of Slanetz and Bartley medium for the enumeration of enterococci in water supplies. The water is filtered through a membrane filter which is then placed on the surface of a well dried plate of the medium. Plates are incubated at 35°C for 4 hours and then at 44–45°C for 44 hours. Membranes are examined, with a hand lens in a good light, and all red or maroon colonies counted as enterococci.

Food samples can be examined for enterococci by the method suggested by the Nordic Committee of Food Analysis[3]. Samples are homogenised and so diluted with physiological saline that only 15–150 colonies grow on each petri dish. Homogenates or dilutions are spread evenly over the agar surface with a glass rod and allowed to soak in. Dishes are inverted and incubated at 35°C for 48 hours, after which typical colonies (pink or dark red, with a narrow whitish border) are counted.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C away from light.

Quality Control
Positive control:
Enterococcus faecalis ATCC® 29212

Negative control:
Escherichia coli ATCC® 25922

Precautions
Count all red, maroon or pink colonies as presumptive enterococci. Not all species reduce TTC therefore pale colonies should not be ignored. Although incubation at 35°C yields a higher count, it allows the growth of organisms which do not conform to the definition of enterococci. Incubation at 44–45°C has a selective effect and produces fewer false-positives. However, the preliminary incubation at 35°C encourages the recovery of stressed organisms.

Although the selective properties of this medium are very good it is advisable to regard the colony count as a presumptive or unconfirmed count. Further identification may be required depending on the scope of the examination.

References

1 Slanetz L. W. and Bartley C. H. (1957) *J. Bact. 74. 591–595.*
2 Burkwall M. K. and Hartman P. A. (1964) *Appl. Microbiol. 12. 18–23.*
3 Nordic Committee on Food Analysis (1968) *Leaflet 68.*

4 Taylor E. W. and Burman N. P. (1964) *J. Appl. Bact. 27. 294–303.*
5 Mead G. C. (1966) *Proc. Soc. Wat. Treat. Exam. 15. 207–221.*
6 Department of Health and Social Security. Report 71 (1982) *The Bacteriological Examination of Drinking Water Supplies. HMSO. London.*

SALMONELLA SHIGELLA AGAR (SS AGAR)

Code: CM99

A differential selective medium for the isolation of Salmonella and some Shigella species from clinical specimens, foods etc.

Formula	gm/litre
'Lab-Lemco' powder	5.0
Peptone	5.0
Lactose	10.0
Bile salts	8.5
Sodium citrate	10.0
Sodium thiosulphate	8.5
Ferric citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	15.0
pH 7.0 $\pm$ 0.2	

Directions
Suspend 63g in 1 litre of distilled water. Bring to the boil with frequent agitation and allow to simmer gently to dissolve the agar. DO NOT AUTOCLAVE. Cool to about 50°C, mix and pour into sterile petri dishes.

Description
SS Agar is a differential, selective medium for the isolation of *Shigella* and *Salmonella* species from pathological specimens, suspected foodstuffs, etc. Gram-positive and coliform organisms are inhibited by the action of the selective inhibitory components brilliant green, bile salts, thiosulphate and citrate.

Thiosulphate in combination with iron also acts as an indicator for sulphide production, which is indicated by blackening in the centres of the colonies.

Technique
Inoculate the medium heavily with the specimen, spreading a portion of the original inoculum in order to obtain well separated colonies on some part of the plate. Incubate for 18 to 24 hours at 35°C; non-lactose fermenters form colourless colonies, whilst occasional resistant coliforms or other lactose fermenters produce pink or red colonies.

In parallel with the SS Agar plate, inoculate a tube of Selenite Broth CM395 enrichment medium, incubate for 12 hours at 35°C, and sub-culture on to another SS Agar plate.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Salmonella enteritidis ATCC® 13076
Shigella sonnei ATCC® 25931

Negative control:
Enterococcus faecalis ATCC® 29212

Precautions
This medium is highly selective and R-strains of shigellae will not grow on it. It is not recommended for the primary isolation of shigellae[1,2].

References
1 Leifson E. (1935) *J. Path. Bact. 40. 581-*
2 Taylor W. I. and Harris B. (1965) *Am. J. Clin. Path. 44. 476-*

SALMONELLA SHIGELLA AGAR (SS AGAR MODIFIED)

Code: CM533

An improved formulation which gives better growth of shigellae and better colony characteristics for salmonellae.

Formula	gm/litre
'Lab-Lemco' powder	5.0
Peptone	5.0
Lactose	10.0
Bile salts	5.5
Sodium citrate	10.0
Sodium thiosulphate	8.5
Ferric citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	12.0
pH 7.3 $\pm$ 0.2	

Directions
Suspend 57g in 1 litre of distilled water. Bring to the boil with frequent agitation, and allow to simmer gently to dissolve the agar. DO NOT AUTOCLAVE. Cool to about 50°C and pour into petri dishes.

Description
Although widely used, SS Agar has been criticised because of excessive inhibition of *Shigella* species.

Investigation has shown that modification to the formulation by alterations to the bile salt mixture, peptone and pH value considerably improve its performance in the growth of shigellae without too much increased growth of commensal organisms.

Salmonella colonies are also larger with improved blackening at the centre.

The change in formulation has reduced the number of gm/litre from 63g to 57g.

Technique
Inoculate the medium heavily with the specimen, spreading a portion of the original inoculum in order

to obtain well separated colonies on some part of the plate. Incubate for 18 to 24 hours at 35°C; non-lactose fermenters form colourless colonies. Occasional resistant coliforms and other lactose fermenters produce pink or red colonies.

In addition to the SS Agar (Modified) plate, inoculate a tube of Selenite Broth Enrichment Medium, CM395. Incubate it for 12 hours at 35°C, and sub-culture on to another SS Agar (Modified) plate.

Colonial Characteristics

Non-Lactose Fermenting Organisms

Salmonella species	Transparent colonies usually with black centres
Shigella species	Transparent colonies
Proteus species *Citrobacter* species	Transparent colonies with grey-black centres

Late-lactose fermenting organisms will develop colonies with pink centres after 48 hours incubation.

Storage conditions and Shelf life
As for SS Agar CM99.

Quality Control
As for SS Agar CM99.

STAPHYLOCOCCUS MEDIUM NO.110

Code: CM145

A selective medium for the isolation and differentiation of pathogenic staphylococci based on salt tolerance, pigmentation, mannitol fermentation and gelatin liquefaction.

Formula	gm/litre
Yeast extract	2.5
Tryptone	10.0
Lactose	2.0
Mannitol	10.0
Sodium chloride	75.0
Dipotassium hydrogen phosphate	5.0
Gelatin	30.0
Agar	15.0
pH 7.1 ± 0.2	

Directions
Suspend 150g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Disperse the precipitate by gentle agitation before pouring.

Description
Staphylococcus Medium No.110 is a selective medium for isolation and differentiation of pathogenic staphylococci (Chapman[12]) on a basis of salt tolerance, pigmentation, mannitol fermentation, and gelatin liquefaction. Pathogenic staphylococci (coagulase-positive) are able to grow on the high-salt mannitol medium to form orange colonies which give positive reactions for acid production and gelatin liquefaction.

Stone[3] suggested that gelatinase activity was indicative of food poisoning strains but Chapman et al.[4] reported that typical food poisoning staphylococci should also produce an orange pigment, be haemolytic, be coagulase-positive, and ferment mannitol. Chapman[5] showed that incubation at 30°C produced deeper pigmentation and no interference with the Stone reaction or with acid production from mannitol – both of the latter being about as intense as at 35°C.

Smuckler & Appleman[6] made Staphylococcus Medium No.110 selective, for the determination of coagulase-positive staphylococci in meat pies containing large numbers of *Bacillus* species, by the addition of sodium azide 0.75mM (4.875 grams per litre).

Staphylococcus Medium No.110 is formulated according to the APHA[7] and AOAC[8] specifications. Carter[9] modified the medium by adding egg yolk (5% v/v SR47) so that the characteristic egg yolk reactions of staphylococci can be seen.

Technique
Streak or smear the Staphylococcus Medium No.110 plate with the specimen and incubate for 43 hours at 35°C or for 48 hours at 30°C. Pigmented colonies are a deep orange colour, whilst non-pigmented colonies are white.

Acid production from mannitol is best demonstrated by adding a drop of 0.04% bromothymol blue indicator to the sites of the individual colonies; yellow indicates acid production.

Gelatin hydrolysis may be demonstrated by adding a drop of a saturated aqueous solution of ammonium sulphate or, preferably, of a 20% aqueous solution of sulphosalicylic acid to an individual colony ('Stone reaction'). A positive 'Stone reaction' is denoted by the presence of a clear zone round gelatinase-producing colonies after 10 minutes' contact with the reagent.

The above reactions may be conveniently performed using short sleeves, 5mm long and 10mm diameter, cut from polythene tubing. The sleeves act as receptacles for the reagents when placed over discrete colonies, and may be stored in 70% alcohol prior to use.

Coagulase tests should not be carried out without first sub-culturing in Nutrient Broth No.2 CM67 or on Blood Agar Base CM55.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923

Negative control:
Escherichia coli ATCC® 25922

Precautions
Enterococcus faecalis may grow on this medium as tiny colonies with slight mannitol fermentation.

The high salt content in Staphylococcus Medium No.110 may interfere with the coagulase reaction. Always sub-culture to a non-inhibitory medium before testing.

References

1 Chapman G. H. (1946) *J. Bact. 51. 409–410.*
2 Chapman G. H. (1952) *J. Bact. 63. 147–150.*
3 Stone R. V. (1935) *Proc. Soc. Exper. Biol. & Med. 33. 185–187.*
4 Chapman G. H., Lieb C. W. and Cumco L. G. (1937) *Food Research 2. 349–367.*
5 Chapman G. H. (1947) *J. Bact. 53. 365–366.*
6 Smuckler S. A. and Appleman M. D. (1964) *Appl. Microbiol. 12. 335–339.*
7 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.*
8 Association of Official Analytical Chemists (1978) *Bacteriological Analytical Manual. 5th Edn. AOAC. Washington DC.*
9 Carter C. H. (1960) *J. Bact. 79. 753–756.*

STAPH/STREP SELECTIVE MEDIUM

For the selective isolation of Staphylococcus aureus and streptococci from clinical specimens or foodstuffs.

BASE MEDIUM

COLUMBIA BLOOD AGAR BASE

Code: CM331

Formula	gm/litre
Special peptone	23.0
Starch	1.0
Sodium chloride	5.0
Agar	10.0
pH 7.3 ± 0.2	

Directions
Suspend 39g in 1 litre of distilled water. Boil to dissolve the medium completely. Sterilise by autoclaving at 121°C for 15 minutes.

STAPH/STREP SELECTIVE SUPPLEMENT

Code: SR70

Vial contents (each vial is sufficient for 500ml of medium)

Nalidixic acid	7.5mg
Colistin sulphate	5.0mg

Directions
Make up and sterilise Oxoid Columbia Blood Agar Base CM331 and cool to 50–55°C. To each 500ml of medium add 25ml Defibrinated Horse Blood SR50 and the contents of one vial of supplement SR70, reconstituted by the addition of 5ml of 95% Ethanol. Mix gently and pour into petri dishes.

COLUMBIA CNA AGAR

A selective medium for Staphylococci and Streptococci of the type described by Ellner[1] and subsequently named Columbia CNA Agar can be made by adding Oxoid Staph/Strep supplement SR70 to Columbia Agar Blood Base CM331.

Columbia CNA Agar can thus be prepared quickly and conveniently as and when required. Because the antibiotics contained in the supplement are freeze-dried they always show optimal activity at the time of use.

The supplemented Columbia Agar is inhibitory to *Staph. albus* and *Micrococcus* species as well as Gram-positive and Gram-negative rods. It suppresses growth of *Proteus, Klebsiella* and *Pseudomonas* species while permitting unrestricted growth of *Staph. aureus*, haemolytic streptococci and enterococci.

Phenylethyl alcohol medium by comparison frequently permits growth of *Proteus* and *Klebsiella* species as well as showing a marked attenuation of the growth of Gram-positive cocci.

Staph/Strep Supplement enables important Gram-positive cocci to be recognised more readily and isolated easily from the mixed bacterial populations contained in many clinical specimens and foods.

Technique
The medium is inoculated in the normal way and incubated aerobically at 35°C for 18 hours.

DO NOT INCUBATE IN CO_2.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923
Streptococcus pyogenes ATCC® 19615

Negative control:
Escherichia coli ATCC® 25922

Precautions
Incubation in a CO_2-enriched atmosphere will cause inhibition of staphylococcal growth[2]. If it is necessary to incubate plates in such an atmosphere then Staph/Strep Supplement SR70 should not be used.

All suspected staphylococcal and streptococcal colonies should be further investigated to confirm their identity. The Staphylase Test DR595 and the Streptococcal Grouping Kit DR585 are useful for these purposes.

References

1 Ellner P. D., Stoessel C. J., Drakeford E. and Vasi F. (1966) *Tech. Bul. Reg. Med. Technol. 36. No. 3.*
2 Morton C. E. G. and Holt H. A. (1989) *Med. Lab. Sci. 46. 72–73.*

STREPTOCOCCUS SELECTIVE MEDIUM

For the selective isolation of streptococci from clinical specimens or foodstuffs.

BASE MEDIUM

COLUMBIA BLOOD AGAR BASE

Code: CM331

Formula	gm/litre
Special peptone	23.0
Starch	1.0
Sodium chloride	5.0
Agar	10.0
pH 7.3 ± 0.2	

Directions
Suspend 39g in 1 litre of distilled water. Boil to dissolve the medium completely. Sterilise by autoclaving at 121°C for 15 minutes.

STREPTOCOCCUS SELECTIVE SUPPLEMENT (COBA)

Code: SR126

Vial contents (each vial is sufficient for 500ml of medium)

Colistin sulphate	5mg
Oxolinic acid	2.5mg

Directions
Reconstitute one vial by the addition of 2ml of sterile distilled water. Aseptically add the contents of the vial to 500ml of sterile Columbia Blood Agar Medium (Columbia Blood Agar Base CM331 plus 5% Defibrinated Horse Blood SR50) cooled to 50°C. Mix gently and pour into sterile petri dishes.

Description
Streptococcus Selective Supplement SR126 is based on the formulation of Petts (COBA Medium)[1] and is recommended for the selective isolation of streptococci of medical and veterinary importance. It replaces Streptococcus Selective Supplement SR74 in the Oxoid product range because of its greater selectivity.

COBA Medium possesses advantages over other media described for selective isolation of streptococci. Agents previously recommended for inhibition of Gram-positive organisms can be shown to have severe effects on streptococci even at subminimal inhibitory concentrations. The antibiotics gentamicin[2,3,4,5,6], amikacin[7], fucidic acid[8], neomycin[9] and cotrimoxazole[10] have all been shown to have adverse effects as have the long established inhibitors crystal violet and sodium azide. Both colistin and oxolinic acid are thermostable and can, if necessary, be stored without refrigeration.

Streptococci are commonly isolated from the upper respiratory tract. They are also often isolated from burns and other sites where frequently there is an abundance of competing organisms. In order to isolate streptococci, especially when present in small numbers, it is necessary to inhibit the competing flora without any adverse effect by the selective agents upon the *Streptococcus* species. The selective agents colistin sulphate (10µg/ml) and oxolinic acid (5µg/ml) have been found to have no inhibitory effect on *Streptococcus* species although amongst Group D organisms *Enterococcus faecalis* colonies are somewhat smaller. The combination of these two selective agents results in total inhibition of Gram-negative organisms and almost all non-streptococcal Gram-positive organisms. A very few staphylococci and coryneform organisms may grow with reduced colony size. The haemolytic reactions on media containing blood are clearly defined, and the colonial size and growth recovery of streptococcal groups A, B, C, D and G and *Strep. pneumoniae* are comparable to that on a non-selective medium. The selective agents can also be used with Islam's Medium (GBS Agar CM755) for the isolation of Group B streptococci without loss of pigmentation occurring.

Technique
1 Prepare the medium from Columbia Blood Agar Base CM331, Streptocccus Selective Supplement S126 and Defibrinated Horse Blood SR50, according to the directions.
2 Inoculate the plates in the normal way and incubate at 35°C overnight in an atmosphere enriched with 5% carbon dioxide or anaerobically.*
3 Confirm that the colonies are streptococci by microscopy, biochemical or serological tests. The Oxoid Streptococcus Grouping Kit DR585 is recommended for this purpose.

* Improved haemolytic reactions are achieved by anaerobic incubation. Gram-positive anaerobic cocci (*Peptostreptococcus* and *Peptococcus* species) would also be selectively isolated under these conditions[1].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Streptococcus pyogenes ATCC® 19615

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions
All suspected streptococcal colonies should be further investigated for confirmation of identity. The Streptococcal Grouping Kit DR585 is useful for this purpose.

References

1 Petts D. N. (1984) *J. Clin. Microbiol. 19. 4–7.*
2 Gray B. M., Pass M.A. and Dillon M. C. Jr. (1979) *J. Clin. Microbiol. 9. 466–470.*
3 Milatovic D. (1981) *J. Clin. Path. 34. 556–588.*
4 Nichols T. and Freeman R. (1980) *J. Clin. Path. 33. 770–773.*
5 Sondag J. E., Morgens R. K., Hoppe J. E. and Marr J. J. (1977) *J. Clin. Microbiol. 5. 397–400.*
6 Waitkins S. A. (1982) *Med. Lab. Sci. 39. 185–188.*
7 Petts D. N., MSc Thesis, University of Surrey.

8 Lowbury E. J. L., Kidson A. J. and Lilly H. A. (1964) *J. Clin. Pathol. 17. 231–235.*
9 Wren M. W. D. (1980) *J. Clin. Pathol. 33. 61–65.*
10 Dykstra M. A., McLoughlin J. C. and Bartlett R. C. (1979) *J. Clin. Microbiol. 9. 236–238.*

STUART TRANSPORT MEDIUM

Code: CM111

A transport medium for fastidious pathogenic organisms.

Formula	gm/litre
Sodium glycerophosphate	10.0
Sodium thioglycollate	0.5
Cysteine hydrochloride	0.5
Calcium chloride	0.1
Methylene blue	0.001
Agar	5.0
pH 7.4 ± 0.2	

Directions
Suspend 16g in 1 litre of distilled water. Bring to the boil to dissolve completely and dispense into screw-capped 7ml bottles. Fill each bottle to the brim, tighten the cap and sterilise by autoclaving at 121°C for 15 minutes. When sufficiently cool to handle, mix by inversion.

Description
This improved medium originally described by Moffett et al.[1] and Stuart et al.[2], is a non-nutritional semi-solid substrate for the preservation of *Neisseria* species and other fastidious organisms during their transport from clinic to laboratory. Originally formulated for the conservation of *Neisseria gonorrhoeae* and *Trichomonas vaginalis*, it may also be used for the transport of other bacteriological specimens. Stuart et al.[2] noted that the transport medium may also be used for *Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus pyogenes* and *Corynebacterium diphtheriae*. Cooper[3] investigated the extension of Stuart's method to the transport of swabs of clinical material containing upper respiratory tract and enteric pathogens. Stuart[4] published an account of his experiences of the medium in a public health bacteriology, whilst Crookes and Stuart[5] used the transport medium in combination with polymyxin for the cultivation of *N. gonorrhoeae.*

Preparation of Charcoal Swabs for use with Transport Medium
1 Prepare swabs by rolling absorbent cotton-wool on wooden sticks.
2 Boil the swabs in a phosphate buffer solution of the following composition:
   Disodium hydrogen phosphate 0.81 grams
   Potassium dihydrogen phosphate 0.18 grams
   Distilled water 100ml
   pH 7.4
3 Immediately dip the swabs into a 1% suspension of charcoal (pharmaceutical grade).
4 Place in cotton-wool plugged test tubes and sterilise in the autoclave at 121°C for 15 minutes. Dry at 100°C to remove any excess moisture.

Transport of Swabs
After collection of the specimen, place the swab in the middle of the bottle of Stuart Transport Medium. Break off the stick, replace the screw cap tightly and transport to the laboratory as soon as possible.

The transport method will allow the isolation of gonococci from approximately 90% of cases of female gonorrhoea, provided the transport period is under 24 hours; for longer periods the method is still useful up to 3 days[2].

In all cases, specimens should be cultivated as soon as possible or stored in the refrigerator if delay is unavoidable. Wilkinson[6] reported successful isolation after as long as six days storage in a refrigerator.

Trichomonas vaginalis remains viable, in the medium, up to 24 hours whilst Cooper[3] has reported the recovery of upper respiratory tract and enteric pathogens after 8 to 12 weeks storage. Stuart et al.[2] successfully used the transport method for the recovery of *H. influenzae, Str. pneumoniae, Str. pyogenes* and *C. diphtheriae* from specimens which had been in transit for 3 to 5 days.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 15–25°C.

Quality Control
Positive control:
Streptococcus pyogenes ATCC® 19615

Negative control:
Uninoculated medium

Precautions
A small amount of blue colour at the top of the bottle indicates oxidation. If this colour extends down into the medium it should be discarded.

Avoid prolonged heating in open flasks, during the preparation of the medium, because thioglycollate is volatile.

Sodium glycerophosphate may be metabolised by some organisms and thus promote their growth.

References
1 Moffett M., Young J.L. and Stuart R. D. (1945) *BMJ. 2. 421–424.*
2 Stuart R. D., Toshach S.R. and Patsula T.M. (1954) *Canad. J. Publ. Hlth 45. 13–83.*
3 Cooper G. N. (1967) *J. Clin. Path. 10. 226–230.*
4 Stuart R. D. (1959) *Pub. Hlth Rep. Wash. 74. 431–438.*
5 Crookes E.M.L. and Stuart R. D. (1959) *J. Path. Bact. 78. 283–288.*
6 Wilkinson A. E. (1955) *J. Med. Lab. Technol. 15. 184–195.*

TERGITOL-7 AGAR

Code: CM793

A selective medium for the detection and enumeration of coliforms.

Formula	gm/litre
Peptone	10.0
Yeast extract	6.0
Meat extract	5.0
Lactose	20.0
Bromothymol blue	0.05
Tergitol-7	0.1
Agar	13.0
pH 7.2 ± 0.2	

TTC Solution (SR148) is supplied as 5ml of filter sterilised 0.05% aqueous solution of tri-phenyltetrazolium chloride (TTC).

Directions
Suspend 54.15 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Dispense in 100ml volumes and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add the contents of 1 ampoule of SR148. Mix well and pour into sterile petri dishes.

Description
Tergitol-7 Agar is a selective and differential medium for the detection and enumeration of coliforms in food and water samples.

Tergitol-7 Agar is based on the formulation described by Chapman[1] and is recommended for the selective isolation and differentiation of the coliform group. The use of Tergitol-7 as a selective agent had been described earlier[2].

The addition of tri-phenyltetrazolium chloride (TTC)[3] allows earlier recognition and identification of *Escherichia coli* and *Enterobacter aerogenes*. This medium has been recommended for examining foodstuffs for faecal contamination[4] and has been successfully used in routine water analysis[5].

Tergitol-7 inhibits Gram positive organisms and minimises the swarming of *Proteus* allowing superior recovery of coliforms. Fermentation of lactose is seen by a change in colour of the pH indicator bromothymol blue. TTC is rapidly reduced to insoluble red formazan by most coliform organisms except *E. coli* and *Enterobacter aerogenes*, thus allowing easy differentiation.

Technique
Inoculate by spreading the sample on the surface of the agar. Incubate at 35°C for up to 24 hours.

Escherichia coli	Yellow colonies with yellow zone. Sometimes with rust coloured centre.
Enterobacter/Klebsiella species	Greenish/yellow colonies
Salmonella species	Red colony with bluish zone
Shigella species	Red colony with bluish zone
Proteus species	Red colony with bluish zone
Pseudomonas species	Red colony with bluish zone
Gram positive bacteria	No growth to slight growth.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Escherichia coli ATCC® 25922

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions
Tergitol-7 Agar is designed for early detection of *Esch. coli* i.e. 6–10 hours incubation[3].

Incubation at 44°C has been recommended[4,5].

References

1 Chapman G. H. (1947) *J. Bact. 53. 504.*
2 Pollard A.L. (1946) *Science 103. 758–759.*
3 Chapman G.H. (1951) *Am. J. Pub. Hlth 41. 1381.*
4 Mossel D. A. A. (1962) *J. Appl. Bact. 25. 20–29.*
5 Kulp W., Mascoli C., Tavshanjian O. (1953) *Am. J. Pub. Hlth 43. 1111–1113.*

TETRATHIONATE BROTH BASE

Code: CM29

Formula	gm/litre
'Lab-Lemco' powder	0.9
Peptone	4.5
Yeast extract	1.8
Sodium chloride	4.5
Calcium carbonate	25.0
Sodium thiosulphate	40.7
pH 8.0 ± 0.2	

Directions
Add 77g to 1 litre of distilled water and bring to the boil. Cool below 45°C and add 20ml of iodine solution. Mix well and tube in 10ml quantities. The prepared base will keep for several weeks at 4°C but should be used soon after the addition of the iodine solution.

Iodine Solution	
Iodine	6 grams
Potassium iodide	5 grams
Distilled water	20ml

Description
Tetrathionate Broth is recommended for the selective enrichment method of isolating *Salmonella typhi* and other salmonellae from faeces, sewage, etc.

Organisms which reduce tetrathionate, such as salmonellae, flourish in the medium whilst many faecal organisms are inhibited[1]. Members of the

Proteus group reduce tetrathionate and may consequently impair the value of this medium for the isolation of salmonellae; this disadvantage of the medium is largely overcome by the addition of 40mg of novobiocin to each millilitre of the incomplete medium before the addition of iodine[2,3].

Technique
Inoculate the broth with about 2 grams of the specimen and mix thoroughly to disperse particulate matter. A loose cotton-wool plug may be passed down through the inoculated medium in order to carry gross particles to the bottom of the tube. Incubate for 12 to 24 hours at 35°C and then sub-culture on Bismuth Sulphite Agar CM201, SS Agar CM99 or Desoxycholate Citrate Agar (Hynes) CM227, etc.

The complete medium (with added iodine) should be used the same day as it is prepared, but the sterilised basal medium will keep for many weeks at 4°C. Jeffries[2] showed that novobiocin, at a concentration of 40µg/ml in the medium, remained stable for at least 48 hours at 35°C, and for one month at room temperature.

This medium is frequently used in parallel with Selenite Broth Base CM395.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium (without iodine solution) at 2–8°C.

Quality Control
Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922

References

1 Knox R., Gell P. G. H. and Pollock M. R. (1942) *J. Path. Bact. 54. 469–483.*
2 Jeffries L. (1959) *J. Clin. Path. 12. 568–571.*
3 Buttiaux R., Catsaras M. and Verdant M. (1961) *Ann. Inst. Pasteur de Lille 12. 13–18.*

TETRATHIONATE BROTH (USA)

Code: CM671

An American formulation which complies with the description given in the US Pharmacopoeia for the enrichment of specimens undergoing examination for salmonellae.

Formula	gm/litre
Casein peptone	2.5
Meat peptone	2.5
Bile salts	1.0
Calcium carbonate	10.0
Sodium thiosulphate	30.0

Directions
Suspend 46 grams in 1 litre of distilled water and bring to the boil. Cool to below 45°C and add 20ml of iodine-iodide solution immediately before use. Mix continuously whilst dispensing 10ml volumes into sterile tubes. Use the complete medium (with added iodine) on the day of preparation.

Iodine-Iodide Solution

Iodine	6 grams
Potassium iodide	5 grams
Distilled water	20ml

Note
The base may be prepared beforehand and kept for several weeks at 4°C. Iodine-iodide solution can then be added at the time of use to the quantity of medium needed.

Description
Tetrathionate Broth USA CM671 complies with the description given in the United States Pharmacopoeia.

Tetrathionate Broth is specified by the 15th edition of Standard Methods for the Examination of Water and Wastewater[2] and Compendium of Methods for the Microbiological Examination of Foods[3] for the enrichment of specimens undergoing examination for salmonellae.

The selectivity of the medium depends on the ability of thiosulphate and tetrathionate in combination to suppress commensal coliform organisms[4]. Organisms which possess the enzyme tetrathionate reductase grow in the medium. *Salmonella* and *Proteus* species possess the enzyme; *Escherichia coli* and shigellae do not.

Proteus can be suppressed by adding 40µg per ml of novobiocin[6] to the incomplete medium before the addition of iodine.

Bile salts are present to inhibit those organisms which do not live in the intestine.

Brilliant Green 0.001% w/v can be added to the broth[1] but it should be remembered that *Salm. typhi* and some other salmonellae are inhibited by this compound.

The role of calcium carbonate is to neutralise the acidic tetrathionate decomposition products.

Technique
Inoculate the broth with 1–2 grams of the specimen and mix thoroughly to disperse the sample.

Incubate at 35°C and sub-culture after 18–24 hours to XLD Agar CM469, SS Agars CM99 or CM533, Bismuth Sulphite Agar CM201, or similar selective/indicator media for salmonella isolation.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the base broth at 2–8°C. Use the medium immediately after adding the iodine solution.

Quality Control
Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922

References

1 United States Pharmacopoeia XXI (1985) *Microbial Limit Tests. Rockville, Md.*
2 American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington DC.*
3 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.*
4 Pollock M. R. and Knox R. (1943) *Biochem. J. 37. 476–481.*
5 Papavassiliou J., Samaraki-Lyberopoulou V. and Piperakis G. (1969) *Can. J. Microbiol. 15. 238–240.*
6 Jeffries L. (1959) *J. Clin. Path. 12. 568–571.*

THIOGLYCOLLATE BROTH USP – ALTERNATIVE

Code: CM391

Used in place of Thioglycollate Medium USP CM173 for testing turbid or viscous products.

Formula	gm/litre
L-cystine	0.5
Sodium chloride	2.5
Glucose	5.5
Yeast extract	5.0
Pancreatic digest of casein	15.0
Sodium thioglycollate	0.5
pH 7.1 ± 0.2	

Directions
Suspend 29 grams in 1 litre of distilled water. Bring to the boil and dissolve the medium completely. Distribute into tubes or bottles and sterilise by autoclaving at 121°C for 15 minutes.

PREPARE FRESHLY OR BOIL AND COOL THE MEDIUM JUST BEFORE USE.

Description
Thioglycollate Medium USP – Alternative, is intended for sterility testing with certain biological products that are turbid or otherwise do not lend themselves readily to culturing in Thioglycollate Medium USP (CM173) because of its viscosity.

The formulation, which omits the agar and resazurin present in Thioglycollate Medium USP, is described in the N.I.H. Memorandum[1] and the U.S. Pharmacopoeia XXI. These omissions make it essential that the medium should be freshly prepared or boiled and cooled within four hours of use.

The containers of choice for the medium are tubes of 20 x 150mm size. The use of 15ml of medium in this tube provides adequate medium for inocula up to 3ml and sufficient thioglycollate to inactivate a mercurial preservative, when present in the inoculum, in a concentration not greater than 0.03% w/v. Other preservatives will need to be inactivated by adequate dilution unless an effective inactivator is used. (See Oxoid Clausen Medium CM353.)

If larger volumes of medium are required, cylindrical or square bottles or containers which provide approximately the same ratio of surface exposed to depth of medium as in the test-tube above, should be used.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Whilst storage of the prepared medium is not recommended, autoclaved volumes of Thioglycollate Medium USP – Alternative, should be held at 20°–30°C in the dark. Storage at lower temperatures increases oxygen absorption.

Quality Control
Positive control:
Candida albicans ATCC® 10231
Bacteroides vulgatus ATCC® 8482
Clostridium sporogenes ATCC® 19404

Negative control:
Uninoculated medium

Precautions
This medium lacks agar and reducing indicator, therefore it is essential that the medium is freshly prepared and used within four hours of preparation. Thioglycollate media should not be reheated more than once because toxic oxygen radicles are formed on reheating.

References

1 N.I.H. (1955) *Memorandum: 'Culture media for sterility tests' 4th Revision.*
2 US Pharmocopoeia XXI (1985) *'Sterility tests'.*

THIOGLYCOLLATE MEDIUM (BREWER)

Code: CM23

An anaerobic medium especially useful for the sterility control of solutions containing mercury preservatives.

Formula	gm/litre
'Lab-Lemco' powder	1.0
Yeast extract	2.0
Peptone	5.0
Glucose	5.0
Sodium chloride	5.0
Sodium thioglycollate	1.1
Methylene blue	0.002
Agar	1.0
pH 7.2 ± 0.2	

Directions
Suspend 20g in 1 litre of distilled water. Bring to the boil, mix well and allow to stand until completely dissolved. Distribute into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Media containing small quantities of agar are liable to separate if cooled rapidly. Tubes of reconstituted and autoclaved medium should be allowed to cool slowly on a wooden surface in a draught-free atmosphere.

Description
This anaerobic medium, developed by Brewer[1,2,3] is used principally for testing the sterility of biological products.

The medium contains a small concentration of methylene blue as an oxidation-reduction indicator. If more than 20% of the uppermost portion of the stored medium has changed to a green colour, anaerobic conditions may be restored by heating in a boiling water bath or steamer for 5 to 10 minutes. This treatment must not be repeated.

Thioglycollate Medium (Brewer) is especially useful for the control of biological solutions containing mercurial preservatives, the toxicity of the latter being neutralised by the thioglycollate.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

The prepared medium should be stored away from light at room temperature.

Quality Control
Positive control:
Bacterioides vulgatus ATCC® 8482
Clostridium perfringens ATCC® 13124

Negative control:
Uninoculated medium.

Precautions
Check the upper portion of the prepared medium before inoculation. If more than one-third of the fluid is oxidised i.e. green coloured, discard the bottle. If one-third or less fluid is oxidised, then heat in boiling water with the cap loosened to drive off oxygen. Cool to room temperature before inoculation. This reheating process can only be carried out once because of the formation of toxic radicles in the medium.

Organisms which ferment glucose and lower the pH to critical levels may not survive in this medium after growth has taken place.

References

1 Brewer J. H. (1940) *JAMA. 115. 598–600.*
2 Brewer J. H. (1940) *J. Bact. 39. 10–13.*
3 Brewer J. H. (1943) *J. Bact. 46. 395–398.*

THIOGLYCOLLATE MEDIUM USP

Code: CM173

A medium for the cultivation of both aerobic and anaerobic organisms in the performance of sterility tests.

Formula	gm/litre
Yeast extract	5.0
Tryptone	15.0
Glucose	5.5
Sodium thioglycollate	0.5
Sodium chloride	2.5
L-cystine	0.5
Resazurin	0.001
Agar	0.75
pH 7.1 ± 0.2	

Directions
Suspend 29.5g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well and cool to room temperature.

Description
This medium is prepared according to the formula specified in the US Pharmacopoeia[1] for the performance of sterility tests. It is suitable for the cultivation of both aerobic and anaerobic organisms. No paraffin or special seal is necessary, nor is an anaerobic jar required for the cultivation of anaerobes. It is well buffered so that acid or alkaline inocula produce negligible alteration in the reaction of the medium. The sodium thioglycollate content of the medium will neutralise the bacteriostatic effect of mercurial compounds used as preservatives in solutions for injection, etc. If the solution undergoing test contains a bacteriostatic substance it is necessary, in order to avoid a false negative result, to establish the bacteriostatic activity of the product by the method described in the US Pharmacopoeia[1]. Thioglycollate Medium USP is also recommended for the cultivation of *Clostridium* species. Sealey[2] found that, of the media tested, Thioglycollate Medium USP gave the best results for the cultivation and maintenance of *Desulfotomaculum nigrificans.*

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium away from light at room temperature.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923
Bacteroides vulgatus ATCC® 8482
Candida albicans ATCC® 10231
Bacillus subtilis ATCC® 6633

Negative control:
Uninoculated medium.

Precautions
If the upper portion of the medium is pink because of oxidation, anaerobic conditions can be restored by reheating for 10 minutes in boiling water or steam. Do not reheat more than once.

If more than one-third of the medium is oxidised then it should be discarded.

Some glucose-fermenting organisms which are able to reduce the pH of the medium to a critical level may not survive in this medium. Early sub-culture is necessary to isolate these organisms.

References

1 US Pharmacopoeia. XXI (1985) *Sterility Testing.*
2 Sealey J. Q. (1951) *Thesis of the University of Texas.*

TINSDALE AGAR BASE

Code: CM487

A medium for the isolation and identification of C. diphtheriae.

Formula	gm/litre
Proteose peptone	20.0
Yeast extract	5.0
Sodium chloride	5.0
L-cystine	0.24
Agar	15.0

pH 8.0 (approx. after addition of supplement)

Directions
Suspend 9g of agar base in 200ml of distilled water. Bring to the boil and dissolve completely. DO NOT AUTOCLAVE. Allow to cool to 50°C and add the rehydrated contents of a vial of Oxoid Tinsdale Supplement SR65. Mix throughly and pour into sterile dishes.

TINSDALE SUPPLEMENT

Code: SR65

Vial contents (each vial is sufficient for 200ml of medium)

Serum	equiv. 20.0ml
Potassium tellurite	0.069g
Sodium thiosulphate	0.085g

Store at 4°C

Directions
To rehydrate the Tinsdale Supplement, add 15ml sterile distilled water aseptically. Rotate the vial, end over end, to dissolve the contents without frothing.

Description
Oxoid Tinsdale Base CM487 is used with Oxoid Tinsdale Supplement SR65 for the primary isolation and identification of *Corynebacterium diptheriae.*

Tinsdale's original agar medium[1] containing serum, tellurite, cystine and formolised blood was formulated to differentiate between *C. diphtheriae* and the diphtheroids found in the upper respiratory tract. This differentiation was based on the ability of *C. diphtheriae* to produce black colonies, surrounded by a brown/black halo, after incubation at 35°C for 48 hours. Diphtheroids do not have this ability. The dark halo is due to the production of H_2S from cystine, interacting with the tellurite salt.

Oxoid Tinsdale Base and Supplement are based on Billings'[2] modification of Tinsdale's Medium, which improved the differential qualities as well as the reproducibility of the medium. Moore and Parsons[3] using Billings' modification confirmed the stability of halo formation on the clear medium and its specificity for *C. diphtheriae* and *C. ulcerans*.

They considered that as the incidence of diphtheria gets smaller, it becomes more essential to have a medium which gives a distinctive, characteristic colony.

Technique
Inoculate the medium to obtain well separated colonies. Stab deep into the agar at intervals in order to initiate browning at an early stage (10–12 hours incubation).

Plates are incubated at 35°C and examined after 24 hours and 49 hours incubation. Growth of *C. diphtheriae* may be inhibited if Tinsdale Agar is incubated in carbon dioxide-enriched air e.g. in a CO_2 incubator.

Browning may be regarded as presumptive evidence of the presence of *Corynebacterium diphtheriae* although 48 hours incubation may be necessary for the recognition of characteristic colonies.

Colonial Characteristics

C. diphtheriae biotype gravis – *C. diphtheriae biotype mitis* *C. ulcerans*	small, shiny black convex colonies with dark brown halos after 24 hours incubation.
C. diphtheriae biotype intermedius –	pale brown colonies which form halos after 36 hours incubation.
Diphtheroids – (*C. pseudodiphtheriticum*)	dark, brown colonies without halos.
Haemophilus, Klebsiella, Neisseria, Staphylococcus and *Streptococcus* species –	minute colonies showing no discoloration of the medium.
Proteus species –	brown-black colonies showing characteristic odour and morphology.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C for not more than 4 days.

Quality Control
Positive control:
C. diphtheriae gravis ATCC® 19409

Negative control:
Uninoculated medium

Precautions
Further tests must be carried out on colonies suspected as *C. diphtheriae*, including cell morphology after sub-culture to Loeffler's Medium and examination for toxin production.

Do not incubate Tinsdale's Agar plates in enhanced CO_2 atmosphere (5–10% v/v).

References

1 Tinsdale G. F. (1947) *J. Path. Bact. 59. 461–464.*
2 Billings E. (1956) *'An Investigation of Tinsdale's Tellurite Medium, its Usefullness and Mechanism of Halo Formation'. Thesis Univ. Michigan.*
3 Moore Mary S. and Parsons Eliz. I. (1958) *J. Infect. Dis. 102. 88–91.*

TODD-HEWITT BROTH

Code: CM189

A medium for the production of antigenic streptococcal haemolysin and the cultivation of streptococci prior to serological grouping.

Formula	gm/litre
Infusion from 450g fat-free minced meat	10.0
Tryptone	20.0
Glucose	2.0
Sodium bicarbonate	2.0
Sodium chloride	2.0
Disodium phosphate	0.4
pH 7.8 ± 0.2	

Directions
Dissolve 36.4g in 1 litre of distilled water. Mix well, distribute into containers and sterilise by autoclaving at 115°C for 10 minutes.

Description
An easily reconstituted, dehydrated modification of the medium originally described by Todd and Hewitt[1] for the production of antigenic streptococcal haemolysin. Fermentation of glucose, which is included as a growth stimulant, would lead to the destruction of haemolysin by the acid produced; consequently, the medium is buffered with sodium bicarbonate and sodium phosphate.

It has been found that inorganic phosphates have a stimulating effect on the growth of pneumococci quite apart from their buffering power.

Todd-Hewitt Broth may be employed as an alternative to serum broth or horse-flesh digest broth, for the cultivation of streptococci prior to serological grouping[2].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Streptococcus pyogenes ATCC® 19615
Streptococcus pneumoniae ATCC® 6303

Negative control:
Uninoculated medium.

Precautions
Streptococcus species grown in Todd-Hewitt Broth and harvested as antigens to raise antibodies, will sometimes carry antigenic material from the broth. This problem must be looked for in the antisera. If detected it would be preferable to use another medium to grow the test streptococci.

References
1 Todd E. W. and Hewitt L. F. (1932) *J. Path. Bact. 35(1). 973–974.*
2 Finegold S. M. and Martin W. J. (1982) *Diagnostic Microbiology. C. V. Mosby Co. St. Louis. USA. p. 645.*

TOMATO JUICE AGAR

Code: CM113

A medium for the cultivation and enumeration of Lactobacillus species.

Formula	gm/litre
Tomato juice (solids from 400ml)	20.0
Peptone	10.0
Peptonised milk	10.0
Agar	12.0
pH 6.1 ± 0.2	

Directions
Suspend 52g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
This is a modification of Kulp's medium for the culture of lactobacilli, and its preparation only differs slightly from the method described by Kulp & White[1]. Tomato Juice Agar is recommended for the cultivation and enumeration of *Lactobacillus* species.

When a more acid medium is required, for the direct plate count of lactobacilli and other organisms from saliva, the reaction of the medium may be adjusted to approximately pH 5.1 by the addition of 1ml of 10% Lactic Acid SR21 to each 100ml of sterilised medium.

Davis[2] compared the morphology of *Lactobacillus* colonies on Oxoid Tomato Juice Agar and other media.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Lactobacillus fermentum ATCC® 9338

Negative control:
Streptococcus mitis ATCC® 9895 (if pH reduced to 5.1)

References
1 Kulp J. W. L. and White V. (1932) *Science 76. 17–18.*
2 Davis G. H. G. (1959) *Lab. Prac. 8(5). 161–167.*

TRICHOMONAS MEDIUM

Code: CM161

A medium for the cultivation of Trichomonas vaginalis.

Formula	gm/litre
Liver digest	25.0
Glucose	5.0
Sodium chloride	6.5
Agar	1.0
pH 6.4 ± 0.2	

Directions
Suspend 37.5g in 1 litre of distilled water. Bring to the

boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Inactivate 80ml of horse serum*, adjust to pH 6.0 and add it to the medium. For diagnostic work, bacterial growth may be suppressed by the addition of 1000 units of penicillin and 500µg of streptomycin per ml of medium or 100µg of chloramphenicol per ml of medium (1 vial of SR78 per 500ml medium).

*Prior to incorporation in the medium, Horse Serum SR35 is inactivated by maintaining at a temperature of 56°C for 30 minutes, and then acidified with N/1 hydrochloric acid to pH 6.0.

Description
A medium based on that of Feinberg & Whittington[1] for the detection of *Trichomonas vaginalis* and *Candida* species. The authors, after a series of investigations on 1,704 genito-urinary specimens, found that *T. vaginalis* would not have been detected in 23.5% of the samples had they not been cultured. Furthermore, out of 747 vaginal specimens, 63% which were positive would have been dismissed as negative without use of the medium.

Stenton[2] noted that the success of an earlier version of the Trichomonas Medium was largely dependent on the choice and percentage of liver incorporated and he selected Oxoid Liver Infusion. Trichomonas Medium has been slightly modified by the incorporation of 0.1% w/v of agar which leads to reduced oxygen tension and consequently more prolific growth of trichomonads. A good growth of both *Trichomonas* and *Candida* may be obtained in mixed culture – the growth of *Candida* species seldom interferes with trichomonads.

Technique
Inoculate Trichomonas Medium and incubate at 35°C for three to five days. At intervals, microscopically examine medium taken from the bottom of the tube. In addition, microscopically examine fresh wet smears of the specimen at the time of initial culture.

The medium is equally suitable for the examination of urethral and vaginal swabs, and urine specimens.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Trichomonas vaginalis ATCC® 30001
Candida albicans ATCC® 10231

Negative control:
Uninoculated medium

References

1 Feinberg J. G. and Whittington Joan M. (1957) *J. Clin. Path. 10. 327–329.*
2 Stenton P. (1957) *J. Med. Lab. Technol. 14. 228–230.*

TRIPLE SUGAR IRON AGAR

Code: CM277

A composite medium for the differentiation of Enterobacteriaceae by three sugar fermentations and hydrogen sulphide production.

Formula	gm/litre
'Lab-Lemco' powder	3.0
Yeast extract	3.0
Peptone	20.0
Sodium chloride	5.0
Lactose	10.0
Sucrose	10.0
Glucose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.3
Phenol red	q.s
Agar	12.0
pH 7.4 ± 0.2	

Directions
Suspend 65g in 1 litre of distilled water. Bring to the boil to dissolve completely. Mix well and distribute. Sterilise by autoclaving at 121°C for 15 minutes. Allow the medium to set in sloped form with a butt about 1 in. deep.

Description
A composite medium for the differentiation of Enterobacteriaceae according to their ability to ferment lactose, sucrose and glucose, and to produce hydrogen sulphide.

Not only does this medium perform most of the functions of Kligler Iron Agar but, in addition, its sucrose content permits the recognition and exclusion of sucrose-fermenting species. These organisms may ferment lactose slowly or not at all during the incubation period, but they attack sucrose readily. Some *Proteus* and other species may give similar reactions to salmonellae and shigellae and it is necessary to distinguish them by their ability to hydrolyse urea. For this reason Triple Sugar Iron Agar should be used in parallel with Urea Broth or Urea Agar.

This medium was formerly considered to be interchangeable with Kligler medium for the detection of hydrogen sulphide producing Enterobacteriaceae. It is now thought that Triple Sugar Iron Agar is not suitable for the detection of hydrogen sulphide production by sucrose-fermenting organisms, such as some *Citrobacter* and *Proteus* species, in which the sucrose fermentation masks the hydrogen sulphide indicator in the medium*[1].

Triple Sugar Iron Agar is recommended for the presumptive identification of colonies or sub-cultures from plating media such as Salmonella Shigella Agar (Modified) CM533, Bismuth Sulphite Agar CM201, Brilliant Green Agar CM263, MacConkey Agar No.3 CM115, or Desoxycholate Citrate Agar (Hynes) CM227.

Technique
The USA techniques are described elsewhere[2,3] but the following is suggested as a simple method:

1 Pick a single colony from the surface of a selective plating medium and smear a MacConkey Agar CM7 plate. Incubate for 18 hours at 37°C and inoculate two separate tubes of media from one single isolated colony:
   (i) Triple Sugar Iron Agar – smear the slope and stab the butt.
   (ii) Urea Broth Base CM71 (with added Urea Solution SR40).
2 Incubate at 35°C.
3 Examine the Urea Broth tube after 5 hours and again after 18 hours incubation. Discard tubes showing a red or pink coloration, which is due to urea hydrolysis by *Proteus* or other organisms.
4 Where there is no urea hydrolysis, examine the Triple Sugar Iron Agar tubes after 18 hours and 48 hours. The following are typical reactions:

Organism	*Butt*	*Slope*	*H_2S*
Enterobacter aerogenes	AG	A	–
Enterobacter cloacae	AG	A	–
Escherichia coli	AG	A	–
Proteus vulgaris	AG	A	+
Morganella morganii	A or AG	NC or ALK	–
Shigella dysenteriae	A	NC or ALK	–
Shigella sonnei	A	NC or ALK	–
Salmonella typhi	A	NC or ALK	+
Salmonella paratyphi	AG	NC or ALK	–
Salmonella enteritidis	AG	NC or ALK	+
Salmonella typhimurium	AG	NC or ALK	+

AG = acid (yellow) and gas formation
A = acid (yellow)
NC = no change
ALK = alkaline (red)
\+ = hydrogen sulphide (black)*
– = no hydrogen sulphide (no black)*
* See note on previous page.

The presumptive evidence so obtained may be confirmed serologically after sub-culturing the organism from the Triple Sugar Iron Agar slope in Nutrient Broth No.2 CM67.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Typical reactions of organisms in Triple Sugar Iron Agar.

Organism	*Slant*	*Butt*	*Gas*	*H_2S*
Escherichia coli ATCC® 25922	A	A	+	–
Proteus vulgaris ATCC® 13315	A	A	+	+
Pseudomonas aeruginosa ATCC® 9027	ALK	ALK	–	–
Salmonella enteritidis ATCC® 13076	ALK	A	+	+

References

1 Bulmash J. M. and Fulton M. (1966) *J. Bact. 88. 1813.*
2 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.*
3 Edwards P. R. and Ewing W. H. (1972) *Identification of Enterobacteriacea. 3rd Edn. Burgess Publishing Co. Minneapolis. USA.*

TRYPTONE BILE AGAR

Code: CM595

A rapid and direct plate method for the enumeration of Escherichia coli in food.

Formula	gm/litre
Tryptone	20.0
Bile salts No.3	1.5
Agar	15.0
pH 7.2 ± 0.2	

Directions
Suspend 36.5g in 1 litre of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and pour 12–15ml of the medium into sterile dishes.

Description
Tryptone Bile Agar CM595 has been developed according to the formulation of Anderson and Baird-Parker[1] for the detection and enumeration of *Escherichia coli* in foods.

It has several advantages over older methods:

1 It is faster.
2 It is less variable.
3 It gives better recovery from frozen samples.
4 It detects anaerogenic and poor lactose-fermenting strains.

The Direct Plating Method (DPM) described by Anderson and Baird-Parker is a modification of that described by Delaney et al[2]. This method, developed for the detection and enumeration of *Esch. coli* in water and food samples, utilises the ability of *Esch. coli* to produce indole from tryptophan at 44°C when grown on a cellulose acetate membrane on plates of Tryptone Bile Agar.

The authors concluded that the formation of indole was a more reliable characteristic for both enterotoxigenic and non-enterotoxigenic strains of *Esch. coli* than lactose fermentation. Ewing[3] found that only 90% of *Escherichia* strains produce acid from lactose within two days, whereas 99% of strains produce indole.

The International Commission on Microbiological Specifications for Foods (CMSF)[4] compared the Most Probable Number (MPN) and the Anderson-Baird-Parker Direct Plating Method (DPM) and concluded that the DPM was preferable to the MPN method of enumeration of *Esch. coli* in raw meats, because of less variability, better recovery from frozen samples, greater rapidity and the smaller quantity of medium needed.

The Direct Plating Method will enumerate both anaerogenic and late lactose-fermenting strains of *Esch. coli* which would be missed by the MPN method. According to Ewing[3] these organisms comprise as many as 10% of *Escherichia* strains.

Holbrook et al.[5] have further modified the Direct Plating Method for detection and enumeration of sublethally damaged cells of *Esch. coli* in frozen, dried, heat processed or acid foods. In this modification the inoculum is applied to a cellulose acetate membrane on Minerals Modified Glutamate Agar and incubated for 4 hours at 37°C. The resuscitation step permits the repair of stressed cells before the transfer of the membrane to a Tryptone Bile Agar plate.

It has been shown that the presence of high levels of fermentable carbohydrates will inhibit the synthesis of tryptophanase[6] and thereby stop indole formation. Holbrook et al. have demonstrated that the resuscitation step reduces the high concentration of sugar present in the inoculum to a level which does not interfere with the production of indole by *Esch. coli* when grown on Tryptone Bile Agar. The resuscitation step should always be carried out when testing dairy or other products containing high concentrations of sugars.

The indole reagent described by Vracko and Sherris[7] was found to be the most suitable, giving the most distinct reaction and reproducibility. The reagent, 5% p-dimethylaminobenzaldehyde in 1N hydrochloric acid is easy to prepare and will not deteriorate when kept for three months in the dark at room temperature.

All indole positive strains give well defined pink colonies when 'stained' using the indole reagent; colonies that do not produce indole are straw coloured.

The growth of indole positive organisms other than *Esch. coli* is inhibited by the selective action of the bile salts and the elevated incubation temperature.

The 'stained' membranes may be 'fixed' by drying in direct sunlight or under a low pressure fluorescent ultra violet lamp with a 'Woods' type filter. When dried the intensity of the staining reaction is improved, and such membranes may be stored for reference.

Technique

Direct Plating Method

1 Prepare plates of Tryptone Bile Agar CM595 and dry the surface.

2 Place a cellulose acetate filter membrane (85mm diameter, 0.45µ pore size), which need not be sterilised, on the surface of the medium. Gently flatten with a sterile spreader to remove trapped air.

3 Prepare the food sample by diluting 1 in 5 or 1 in 10 with 0.1% (w/v) sterile Peptone Water CM9 and homogenise in a 'Stomacher' or a laboratory blender[8].

4 Pipette 0.5 or 1.0ml of the homogenate on to the membrane and spread over the surface with a sterile glass spreader.

5 Allow the homogenate to soak in and incubate plates stacked, not more than three high, with lids uppermost, for 18–24 hours in a water jacketed incubator at 44°C (±1°).

6 Remove the plates from the incubator and pipette 1–2ml of the indole reagent into each labelled lid.

7 Lift the membrane with a pair of forceps from the plate and lower on to the reagent.

8 Place the stained membranes in direct sunlight or under a low pressure uv lamp for 5–10 minutes. Indole positive colonies are stained pink.

9 Multiply the number of pink colonies by the dilution factor and express the result as the number of *Esch. coli* per gram of food.

10 The 'stained' membrane may be 'fixed' by prolonged drying in direct sunlight or under a uv lamp, and kept for reference.

Resuscitation Procedure

1 Preparation of Minerals Modified Glutamate Agar plates.

Make up 1 litre of Minerals Modified Glutamate Medium CM607 and add 12g of Agar No.1 L11. Bring gently to the boil until dissolved completely and sterilise by autoclaving at 116°C for 10 minutes. Cool to 50°C and pour 12–15ml of the medium into sterile dishes.

2 Place a cellulose acetate filter membrane on to the well dried surface of a plate of Minerals Modified Glutamate Agar. Gently flatten with a sterile spreader to remove trapped air.

3 Prepare the food sample by diluting 1 in 5 or 1 in 10 with 0.1% (w/v) Peptone Water CM9 and homogenise in a 'Stomacher' or a laboratory blender.

4 Pipette 0.5 or 1.0ml of the homogenate on to the membrane and spread completely over the surface with a sterile glass spreader.

5 Allow the homogenate to soak in, and incubate the plates with the lids uppermost in piles of not more than three for 4 hours at 35°C.

6 Transfer the membrane filter from the plate using sterile forceps and gently lower on to the dried surface of a Tryptone Bile Agar plate.

7 Incubate the plates as described for the Direct Plating Method, strain, and count the number of pink indole positive colonies.

If required the unstained plates may be placed in the refrigerator overnight and the indole test carried out the following morning.

Indole Reagent
5% p-dimethylaminobenzaldehyde in 1N hydrochloric acid.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control
Stain colonies on the membrane filter with indole reagent.

Positive control:
Escherichia coli ATCC® 25922

Negative control:
Enterobacter aerogenes ATCC® 13048

References

1 Anderson J. M. and Baird-Parker A. C. (1975) *J. Appl. Bact. 39. 111–117.*
2 Delaney J. E., McCarthy J. A. and Grasso R. J. (1962) *Wat. Sewage Works. 109. 289.*
3 Ewing W. H. (1972) *COC Atlanta, US Dept. of Health, Education & Welfare.*
4 International Commission on Microbiological Specifications for Foods (1979) *Can. J. Microbiol. 25. 1321–1327.*
5 Holbrook R., Anderson J. M. and Baird-Parker A. C. (1980) *Food Technol. in Aust. 32. 78–83.*
6 Clarke P. H. and Cowen S. T. (1952) *J. Gen. Microbiol. 6. 187–197.*
7 Vracko R. and Sherris J. C. (1963) *Amer. J. Clin. Path. 39. 429–432.*
8 Sharpe A. N. and Jackson A. K. (1972) *Appl. Microbiol. 24. 175–178.*

TRYPTONE BILE X-GLUCURONIDE MEDIUM (TBX)

Code: CM945

A selective, chromogenic medium for the detection and enumeration of Escherichia coli in food.

Formula

	gm/litre
Tryptone	20.0
Bile Salts No. 3	1.5
Agar	15.0
X-glucuronide	0.075
pH 7.2 ± 0.2	

Directions
Suspend 36.6g of TBX Medium CM945 in 1 litre of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and pour the medium into sterile petri dishes.

Dry the surface of the medium in the prepared plates. Prepare the food sample by diluting 1 in 5 or 1 in 10 (as appropriate) with 0.1% (w/v) sterile Peptone Water CM9, and homogenise in a stomacher or a laboratory blender.

Pipette 0.5ml or 1.0ml (as appropriate) of the homogenate on to the plate and spread over the surface with a sterile glass spreader. Incubate plates for 4 hours at 30°C then 18 hours at 44°C.

Multiply the number of blue/green colonies by the dilution factor and express the result as the number of *E. coli* per gram of food.

Description
TBX Medium is based on Tryptone Bile Agar CM595. Tryptone Bile Agar was originally formulated to improve on earlier methods used to detect *E. coli* in foods[2] in terms of speed, reliability, better recovery from frozen samples and the detection of poor lactose fermenters.

TBX Medium builds on these advantages through the addition of a chromogenic agent – X-glucuronide – which detects glucuronidase activity. This is the same enzyme detected by MUG reagent[3], and has been shown to be highly specific for *E. coli*[4]. However, approximately 3–4% of *E. coli* are glucuronidase negative, notably *E. coli* O157 strains[5].

Unlike MUG, where the flurophore leaches out of the cell into the surrounding agar, the released chromophore in TBX Medium is insoluble and accumulates within the cell. This ensures that coloured target colonies are easy to identify.

Most *E. coli* strains can be differentiated from other coliforms by the presence of the enzyme glucuronidase. The chromogen in TBX Medium is 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide (X-glucuronide), and is targeted by this enzyme. *E. coli* cells are able to absorb this complex intact and intracellular glucuronidase splits the bond between the chromophore and the glucuronide. The released chromophore is coloured and builds up within the cells, causing *E. coli* colonies to be coloured blue/green.

Storage conditions and Shelf life
TBX Medium CM945 should be stored tightly capped in the original container at 10°C–25°C. When stored as directed, the medium will remain stable until the expiry date printed on the bottle.

Quality Control
Positive control:
Escherichia coli ATCC® 25922 – blue/green colonies

Negative control:
Klebsiella pneumoniae ATCC® 11228 – colourless colonies

Precautions
TBX Medium CM945 should only be used for *in vitro* diagnostic purposes.

Do not use beyond the stated expiry date, or if the product is caked, discoloured or shows any sign of deterioration.

References
1 Gross R.J. and Rowe B. (1985) *J. Hyg. Camb. 95. 513–550.*
2 Anderson J.M. and Baird-Parker A.C. (1975) *J. Appl. Bact. 39. 111–117.*
3 Feng P.C.S. and Hartmann P.A. (1982) *Appl. Environ. Microbiol. 43. 1320–1329.*
4 Hansen W. and Yourassowsky E. (1984) *J. Clin. Microbiol. 20. 1177–1179.*
5 Ratnam S., March S.B., Almed R., Bezanson G.S. and Kasatiya S. (1988) *J. Clin. Microbiol. 26. 2006–2012.*

TRYPTONE GLUCOSE EXTRACT AGAR

Code: CM127

For the plate count of water, dairy products and for the detection of thermophilic organisms.

Formula	gm/litre
'Lab-Lemco' powder	3.0
Tryptone	5.0
Glucose	1.0
Agar	15.0
pH 7.0 ± 0.2	

Directions
Suspend 24g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring. When the dilution of the original specimen is greater than 1 in 10, add 10ml of sterile 10% solution of Skim Milk Powder L31 per litre.

Description
This medium, of American origin, is recommended for the plate count of water and dairy products[1,2]. It can also be used for the detection of thermophilic bacteria in dairy products. The Standard Methods Committee of the American Public Health Association recommended that sterile milk should be added to this medium only when the dilution of the original specimen was greater than 1 in 10. The addition of 10ml of a sterile 10% solution of Skim Milk Powder L31 per litre is suitable for this purpose. It is essential that this medium is not overheated during sterilisation.

See also Plate Count Agar CM325.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Compare with previous lot/batch of medium using samples of pasteurised and unpasteurised milk.

References
1 American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington DC.*
2 American Public Health Association (1972) *Standard Methods for the Examination of Dairy Products. 13th Edn. APHA Inc. Washington DC.*

TRYPTONE SOYA AGAR

Code: CM131

A general purpose medium for the growth of a wide variety of organisms.

Formula	gm/litre
Tryptone	15.0
Soya peptone	5.0
Sodium chloride	5.0
Agar	15.0
pH 7.3 ± 0.2	

Directions
Add 40g to 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A general purpose agar medium, containing two peptones, which will support the growth of a wide variety of organisms. It is suitable for the cultivation both of aerobes and anaerobes, the latter being grown either in deep cultures or by incubation under anaerobic conditions. The medium may also be used as a blood agar base – for this purpose 7% of sterile blood should be added to the sterile molten medium which has been cooled to approximately 45°C. Tryptone Soya Agar can also be used for the preparation of 'chocolate' agar.

Since Tryptone Soya Agar contains no added carbohydrate it may be used, with added blood, in the determination of haemolysis.

Horse blood agar plates prepared with Oxoid Tryptone Soya Agar are used for the colicine typing of *Shigella sonnei*[1,2,3,4].

The Oxoid medium has also been used as a replacement for yeastrel-milk agar plates in the Lisboa test[5] and for bacterial counts on eviscerated poultry[6].

When supplemented with 0.7g lecithin and 5g Polysorbate (Tween 80) per litre of Tryptone Soya Agar, the medium can be used as Microbial Content Test Agar for testing quaternary ammonium compounds[7].

Tryptone Soya Agar is recommended as a reference medium when testing selective media, to measure the degree of inhibition[8].

A medium for isolation of *Bacteroides gracilis* is prepared from Tryptone Soya Agar by adding formate, fumarate and nitrate. The medium is made selective using nalidixic acid and teicoplanin[9].

Enchanced haemolysis agar (EHA) used to improve detection of *Listeria monocytogenes* when present amongst other listeriae has been modified to optimise its performance by substituting Tryptone Soya Agar for Columbia Agar in the original formulation[10].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium at 2–8°C.

Quality control
Positive control:
Staphylococcus aureus ATCC® 25923
Streptococcus pyogenes ATCC® 19615

Negative control:
Uninoculated plate.

Precautions
It should be noted that haemolytic reactions of streptococci on Tryptone Soya Agar can vary according to the origin of the blood e.g. horse or sheep. Tryptone Soya Agar designed for sheep blood show significant differences when used with horse blood and vice versa.

References

1 Abbott J. D. and Graham J. M. (1961) *Mon. Bull. Min. Hlth Pub. Hlth Lab. Serv. 20. 51–58.*
2 Barrow G. I. and Ellis C. (1962) *Mon. Bull. Min. Hlth Pub. Hlth Lab. Serv. 21. 141–147.*
3 Cooke G. T. and Daines C. F. (1964) *Mon. Bull. Min. Hlth Publ. Hlth Lab. Serv. 23. 81–85.*
4 Gillies R. R. (1964) *J. Hyg. Camb. 62. 1–9.*
5 Mitchell T. G. (1964) *J. Appl. Bact. 27. 45–52.*
6 Barnes Ella M. and Shrimpton D. H. (1958) *J. Appl. Bact. 2. 313–329.*
7 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.*
8 Anon. (1987) *J. Food Microbiol. 5. 291–296.*
9. Lee K., Baron E.J., Summanen P. and Finegold S. (1990) *J. Clin. Microbiol.* 28. 1747–1750.
10. Beumer R.R., te Giffel M.C. and Cox L.J. (1997) *Lett. Appl. Microbiol.* 24. 421–425.

TRYPTONE SOYA BROTH – SOYBEAN CASEIN DIGEST MEDIUM USP

Code: CM129

A highly nutritious general purpose medium for the growth of bacteria and fungi.

Formula	gm/litre
Pancreatic digest of casein	17.0
Papaic digest of soybean meal	3.0
Sodium chloride	5.0
Dibasic potassium phosphate	2.5
Glucose	2.5
pH 7.3 $\pm$ 0.2	

Directions
Add 30g to 1 litre of distilled water, mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
A highly nutritious versatile medium which is recommended for general laboratory use. Due to the inclusion of both Tryptone and Soya Peptone, the medium will support a luxuriant growth of many fastidious organisms without the addition of serum, etc.

Technique

Aerobic Cultivation.
Tryptone Soya Broth may be used for the cultivation of aerobes and facultative anaerobes, including some fungi. Cultures should be examined at frequent intervals, as maximum growth is reached earlier than with less nutritious media and the phase of decline consequently begins sooner.

Anaerobic Cultivation
The addition of a small amount of agar (e.g. up to one agar tablet CM49 to every 100ml of reconstituted Tryptone Soya Broth, prior to sterilisation) renders the broth suitable for the cultivation of obligatory anaerobes, such as *Clostridium* species. For this purpose, the broth (with added agar) should be used soon after sterilisation, or, heated and cooled just before inoculation.

Blood Culture
The superior growth-promoting properties of Tryptone Soya Broth make it especially useful for the isolation of organisms from blood or other body fluids. Anticoagulants such as 'liquoid'* (sodium polyanethyl sulphonate) or sodium citrate may be added to the broth prior to sterilisation. Five to 10ml of blood may be added to 50ml of medium.

Tryptone Soya Broth is especially suitable for the tube dilution method of antibiotic susceptibility testing.

Oxoid laboratory tests have shown that Tryptone Soya Broth has a greater ability to resuscitate heated spores of *Bacillus stearothermophilus* than Dextrose Tryptone Broth. Tryptone Soya Broth is recommended in the USP XXI (Soybean Casein Digest Medium) for the recovery of organisms after sterilisation processes[1]. A positive result is revealed after 24–48 hours incubation at 55°C, by a heavy growth of organisms causing turbidity in the broth.

Selective Culture Media
Tryptone Soya Broth is used in food bacteriology as the basal medium to which a variety selective agents are added for selective enrichment of *Staphylococcus aureus*[2] and *Escherichia coli* O157[3].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at room temperature.

Quality Control
Positive control:
Streptococcus pneumoniae ATCC® 6303
Staphylococcus aureus ATCC® 25923

Negative control:
Uninoculated medium.

References

1 US Pharmacopoeia. XXI. (1985) *Bethesda MD.*
2 *Compendium of Methods for the Microbiological Examination of Foods. 3rd edition. Vanderzant C. and Splittstoesser D.F. (eds). APHA. Washington DC.*
3 *Practical Food Microbiology. Roberts D., Hooper W and Greenwood M. (Eds). Public Health Laboratory Service, London 1995.*

*Roche Products Ltd. Welwyn Garden City, Herts.

TRYPTONE WATER

Code: CM87

A liquid medium for the production of indole by micro-organisms.

Formula	gm/litre
Tryptone	10.0
Sodium chloride	5.0
pH 7.5 ± 0.2	

Directions
Dissolve 15g in 1 litre of distilled water and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Tryptone Water is a good substrate for the production of indole because of its high content of tryptophan and it is more reliable than Peptone Water for this purpose. The ability of certain organisms to break down the amino-acid tryptophan with formation of indole is an important property which is used for the classification and identification of bacteria[1,2].

Technique
Kovac's reagent:

paradimethylaminobenzaldehyde	5 grams
amyl alcohol	75ml
concentrated hydrochloric acid	25ml

1 Inoculate tubes of Tryptone Water, and incubate for 24–48 hours at 35°C.
2 Add 0.2ml of Kovac's reagent and shake. Allow to stand for 10 minutes and observe the result.

A dark red colour in the amyl alcohol surface layer constitutes a positive indole test; no change in the original colour of the reagent constitutes a negative test.

Ehrlich reagent:

paradimethylaminobenzaldehyde	4 grams
absolute alcohol	380ml
concentrated hydrochloric acid	80ml

1 Inoculate tubes of Tryptone Water, and incubate for 24–48 hours at 35°C.
2 Withdraw a small portion of the culture and add an equal volume of the reagent. Shake the mixture and allow to stand for a few minutes; a rose colour indicates the presence of indole. If indole is present, this colour change may be accelerated by the addition of saturated potassium persulphate solution.

As an alternative, extract the indole by shaking the cultures with 1ml of ether, allow the mixture to stand for a few minutes and then add 0.5ml of Ehrlich reagent.

Incubation at 44°C for 24 hours has the specific advantage of detecting *Escherichia coli*, as this is the only organism present in water capable of producing indole at this temperature[3].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at room temperature.

Quality Control
Positive control:
Escherichia coli ATCC® 25922

Negative control:
Enterobacter aerogenes ATCC® 13048

References

1 American Public Health Association(1980) *Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington DC.*
2 Farmer J. J. III et al. (1985) *J. Clin. Microbiol. 21. 46–76.*
3 DHSS. Report 71 (1982) *The Bacteriological Examination of Drinking Water Supplies. HMSO. London.*

TRYPTOSE BLOOD AGAR BASE

Code: CM233

A highly nutritious medium specially developed for the preparation of a blood agar which will support the growth of fastidious organisms.

Formula	gm/litre
Tryptose	10.0
'Lab-Lemco' powder	3.0
Sodium chloride	5.0
Agar	12.0
pH 7.2 ± 0.2	

Directions
Suspend 30g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

For blood agar, cool the basal medium to 45–50°C and add 7% of sterile blood. Mix thoroughly, taking care to avoid incorporation of air bubbles, and dispense into petri dishes or other containers.

Description
A highly nutritious medium specially developed by Casman[1,2] for the preparation of a blood agar which will support the growth of many fastidious organisms.

The original formulation included 0.3g dextrose per litre which interfered with the haemolytic reactions. It is now used without dextrose as a standard medium[3].

Tryptose Blood Agar Base with added blood gives good haemolytic reactions and without blood it will sustain good to excellent growth of many demanding organisms. However, to improve the growth of certain organisms e.g. *Neisseria meningitidis* and *Streptococcus pneumoniae*, the addition of 1g of yeast extract (L21) to one litre of Tryptose Blood Agar Base can be made.

Technique
Lightly inoculate the surface of the medium and incubate aerobically at 35°C for 18–24 hours in 5–10% carbon dioxide (if necessary) or incubate anaerobically at 35°C for 48 hours to enhance haemolysis and to restrict unwanted commensal growth.

Carry out microscopy and other identification tests on the isolated colonies.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium at 2–8°C.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923
Streptococcus pyogenes ATCC® 19615

Negative control:
Uninoculated medium.

References

1 Casman E. P. (1942) *J. Bact. 43. 33–37.*
2 Casman E. P. (1947) *Amer. J. Clin. Path. 17. 281–282.*
3 American Public Health Association (1970) *Diagnostic Procedures and Reagents. 5th Edn. APHA Inc. New York.*

TRYPTOSE PHOSPHATE BROTH

Code: CM283

A buffered glucose broth for the cultivation of fastidious bacteria.

Formula	gm/litre
Tryptose	20.0
Glucose	2.0
Sodium chloride	5.0
Disodium hydrogen phosphate	2.5
pH 7.3 ± 0.2	

Directions
Dissolve 29.5g in 1 litre of warm distilled water and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

For the cultivation of anaerobes, if the reconstituted medium has been stored prior to use, remove dissolved oxygen by placing the tubes in a boiling water bath for 15 minutes and cool without agitation before inoculation.

Description
A buffered dextrose broth for use as an adjuvant to tissue culture media and for the cultivation of fastidious bacteria. Ginsberg et al.[1] maintained tissue cultures of HeLa cells for at least 10 days in a mixture of Tryptose Phosphate Broth 15–25%, Scheren maintenance solution 67.5–77.5% and chicken serum 7.5%. The cells increased 3–5 fold in number during this period, smaller quantities of ARD, AD and type 1 poliomyelitis virus could be detected and more ARD virus could be propagated in HeLa cells in the Tryptose Phosphate.

Broth supplemented medium.
Tryptose Phosphate Broth is recommended for the cultivation of streptococci, pneumococci, meningococci and other fastidious organisms. Tryptose Phosphate Broth with added agar and sodium azide is recommended for the isolation of pathogenic streptococci from cheese and other dairy products[2,3].

Tryptose Phosphate Broth with added agar is also recommended by the American Public Health Association for the examination of throat swabs and blood for *Streptococcus pneumoniae* and as a growth medium for pneumococci prior to the bile solubility test.

Tryptose Phosphate Agar Broth may also be employed for the emulsification of cheese prior to the plate isolation method for *Brucella* species[2].

The small proportion of agar (0.1–0.2%) necessary for the above methods may be most conveniently added to the Oxoid broth before sterilisation, in the form of Oxoid Agar Tablets CM49 (one tablet per 100ml).

Technique

***Examination of throat swabs for S. pneumoniae*[4]**
1 Prepare Tryptose Phosphate Broth in the usual manner but add a small amount of agar by dissolving 10 Agar Tablets CM49 per litre of broth before autoclaving.
2 Place a pharyngeal or sputum swab in 3ml of Tryptose Phosphate (Agar) Broth and incubate for 2 hours at 35°C. If sufficient pneumococci are present, type directly or preferably employ the culture for mouse inoculation.

***Examination of cheese for Lancefield Group A streptococci*[2]**
1 Heat 25ml of Tryptose Phosphate (Agar) Broth (prepared as above) to 45°C.
2 Immediately emulsify 5 grams of cheese in the broth, using a heavy sterile glass rod. Plate suitable dilutions of the emulsion on Tryptose Agar plates containing 1.5% agar and 0.04% of sodium azide.

Alternatively, add sterile aqueous sodium azide to the emulsion to give a final concentration of 2.5%, incubate for 12–14 hours at 35°C, shake, plate on Tryptose Agar plates containing 1.5% agar and 0.04% of sodium azide.

The sodium azide suppresses the growth of most bacteria except streptococci and some lactobacilli.

Blood Culture
1 Add up to 10ml of blood to 150ml of Tryptose Phosphate Broth in a 300ml flask or bottle.
2 Incubate and sub-culture on to other media in the usual manner, according to the exact purpose of the investigation.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Streptococcus pneumoniae ATCC® 6303
Neisseria meningitidis ATCC® 13090

Negative control:
Uninoculated medium

References

1 Ginsberg H. S. et al. (1955) *Proc. Soc. Exper. Biol. Med. 89. 66–71.*
2 American Public Health Association (1953) *'Standard Methods for the Examination of Dairy Products' 10th ed., APHA Inc., New York, pp. 179, 180, 181.*
3 Newman R. W. (1950) *J. Milk Food Tech. 13. 226–233.*
4 American Public Health Association (1953) *'Diagnostic Procedures and Reagents' 4th ed., APHA Inc., New York, p. 141.*

UNIVERSAL BEER AGAR

Code: CM651

For the isolation of beer spoilage organisms.

Formula	gm/litre
Peptonised milk	15.0
Yeast extract	6.1
Glucose	16.1
Tomato supplement	12.2
Dipotassium hydrogen phosphate	0.31
Potassium dihydrogen phosphate	0.31
Sodium chloride	0.006
Ferrous sulphate	0.006
Manganese sulphate	0.006
Magnesium sulphate	0.12
Agar	12.0
pH 6.1 ± 0.2	

Directions
Suspend 62g in 750ml of distilled water and bring to the boil to dissolve completely. Add 250ml beer, without degassing, to the hot medium and mix gently. Distribute into final containers and sterilise by autoclaving at 121°C for 10 minutes.

Description
Universal Beer Agar is presented as a basal medium to which beer alone or beer and cycloheximide may be added for the detection and culture of microbial contaminants in beer. The medium is based on the formula developed by Kozulis and Page[1], who recommended that beer must be incorporated in the medium in order to increase selectivity by stimulating the growth of beer spoilage organisms. The presence of hop constituents and alcohol eliminates many airborne contaminants not originating in pitching yeasts, wort or beer; thus minimising false positive results.

Oxoid Universal Beer Agar supports the growth of *Lactobacilli*, *Pediococci*, *Acetobacter*, *Zymomonas* species and wild yeast strains which may be found infecting the pitching yeast, the cooled wort, or during fermentation or storage of the finished beer.

Technique
The presence of microbial spoilage organisms in pitching yeast, the cooled wort or beer in storage may be detected and enumerated using Universal Beer Agar. Either direct surface plating or pour plate techniques with serial dilutions of the sample can be employed. Plates are incubated both aerobically to detect *Acetobacter* species and anaerobically to detect the micro-aerophilic *Lactobacilli* and *Pediococci* species as well as the anaerobic *Zymomonas sp.* Plates are incubated at 28–30°C for three days and examined daily.

To increase the differentiation of the colonies, Bromocresol Green (20mg/litre) and powdered chalk (3g/litre) may be added to the medium before sterilisation[2]. Zones of decolorisation are seen around *Pediococcus* and some *Lactobacillus* colonies.

The addition of cycloheximide at 0.001 grams/litre to suppress yeast growth gives a medium that is selective for the detection of bacterial contaminants in yeast cultures.

Storage conditions and Shelf Life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Acinetobacter calcoaceticus ATCC® 19606
Lactobacillus fermentum ATCC® 9338

Negative Control:
Saccharomyces cerevisciae ATCC® 9763
(when cycloheximide is added to the medium)

Precautions
When handling cycloheximide observe the precautions to be taken under HAZARDS page 2–7.

References
1 Kozulis J. A. and Page H. E. (1968) *Proc. Am. Soc. Brew. Chem. 52–58.*
2 Boatwright J. and Kirsop B. H. (1976) *J. Inst. Brew. 82. 343–346.*

UREA AGAR BASE

Code: CM53

An agar base for the preparation of Christensen's medium to detect rapid urease activity of the Proteae and non-rapid urease activity of some Enterobacteriaceae.

Formula	gm/litre
Peptone	1.0
Glucose	1.0
Sodium chloride	5.0
Disodium phosphate	1.2
Potassium dihydrogen phosphate	0.8
Phenol red	0.012
Agar	15.0
pH 6.8 ± 0.2	

Directions
Suspend 2.4g in 95ml of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 115°C for 20 minutes. Cool to 50°C and aseptically introduce 5ml of sterile 40% Urea Solution SR20. Mix well, distribute 10ml amounts into sterile containers and allow to set in the slope position.

Description
Urea Agar Base is recommended for the preparation of Christensen medium[1] for the detection of rapid urease activity of the urease-positive Proteae. The urea medium may be used for the detection of urea hydrolysis by some other Enterobacteriaceae but the incubation period is much longer 24–48 hours.

Technique
Heavily inoculate the surface of a Urea Agar slope with a pure culture of the organism to be tested. When inoculated with urease-positive Proteae the reaction is usually complete after 3 to 5 hours at 35°C: urease-producing organisms hydrolyse the urea to form ammonia, and the medium changes to purple-red.

40% Urea Solution is supplied, as a sterile solution in ampoules, for the convenient preparation of this medium.

Storage conditions and Shelf Life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Proteus vulgaris ATCC®13315

Negative control:
Escherichia coli ATCC®25922

Precautions
The alkaline reaction produced in this medium after prolonged incubation may not be caused by urease activity. Check using medium without urea.

Do not heat or reheat the medium because urea decomposes very easily.

For the detection of urease-positive Proteae the reaction must be read within the first 2–5 hours of incubation.

Reference
1 Christensen W. B. (1946) *J. Bact. 52. 461–466.*

UREA BROTH BASE

Code: CM71

A liquid version of Christensen's medium for the differentiation of urease-producing Enterobacteriaceae.

Formula	gm/litre
Peptone	1.0
Glucose	1.0
Disodium phosphate	1.2
Potassium dihydrogen phosphate	0.8
Sodium chloride	5.0
Phenol red	0.004
pH 6.8 ± 0.2	

Directions
Add 0.9g to 95ml of distilled water. Sterilise by autoclaving at 115°C for 20 minutes. Cool to 55°C and aseptically introduce 5ml of sterile 40% Urea Solution SR20. Mix well and distribute 10ml amounts into sterile containers.

Description
This is a liquid modification of Christensen medium[1]. The modification is suitable for the differentiation of urease-producing organisms from members of the *Salmonella* and *Shigella* groups, during the routine examination of rectal swabs and faeces. Maslen noted that in the routine examination of faeces for *Salmonella* and *Shigella* organisms many non-lactose-fermenting colonies isolated were later found to belong to the urease-positive Proteae. He evolved this medium as a means whereby the latter organisms could be rapidly detected and eliminated – thus saving a considerable amount of time and media. Maslen claimed that the advantages of the fluid medium were:

1 A Pasteur pipette could be used to inoculate other diagnostic media.
2 Rapid growth ensued and it was possible to discern a clear-cut positive reaction within two to five hours at 35°C.
3 It was easier to detect any contamination during storage.

Technique
For the examination of faeces, specimens are cultured in enrichment and selective media in the usual manner. Discrete colonies are then picked off the surface of the solid selective media.

Inoculate tubes of Urea Broth with single colonies of non-lactose-fermenting organisms and incubate for 2 to 6 hours at 35°C. (Maslen states that the cultures should be incubated in a water bath in order to obtain the highest proportion of positive reactions within 5 hours.) Regard all organisms producing a pink coloration in the medium (i.e. due to the alkalinity caused by urea hydrolysis) as not belonging to the *Salmonella* or *Shigella* groups, and discard.

Inoculate all cultures showing no colour change (i.e. no urea hydrolysis) into 'sugar' peptone waters (Andrade Peptone Water CM61) plus the appropriate carbohydrate and on to a Blood Agar Base CM55 slope.

Incubate the new cultures, together with the Urea Broth, until the next morning. No further examination is necessary if the urea tube now shows an alkaline reaction (pink colour), otherwise continue the diagnostic tests – including slide agglutinations from the Blood Agar Base culture, if necessary.

Storage conditions and Shelf Life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Proteus vulgaris ATCC®13315

Negative control:
Escherichia coli ATCC®25922

Precautions
It is preferable that the medium be used on the day of preparation. If not, examine the tubes carefully to ensure sterility.

After overnight incubation other members of the Enterobacteriaceae may show alkaline reactions.

Reference
1 Maslen L. G. C. (1952) *Brit. Med. J. 2. 545–546.*

VIOLET RED BILE AGARS

A guide to the choice of an appropriate medium.

Media containing bile and the dye violet red used in the examination of foods are based on the medium developed by MacConkey for detection of bile-tolerant Gram-negative bacteria. Violet Red Bile Agar CM107 (VRBA) contains lactose as a fermentable carbohydrate which enables the medium to be used for the detection of lactose-fermenting bacteria and differentiation of the group of bacterial genera known as coliforms or coli-aerogenes bacteria from non-lactose-fermenting organisms. Violet Red Bile Glucose Agar CM485 (VRBGA) differs from VRBA only by substitution of glucose for lactose. By definition all members of the Enterobacteriaceae ferment glucose and grow as purple-red 'positive' colonies.

VRBA was used routinely during the years 1925 to 1935 to monitor the efficacy of milk pasteurisation and milking parlour hygiene. It is still considered to be a useful medium for testing the hygienic status of water and milk. All Gram-negative bacteria capable of growth on bile-containing media and which ferment lactose are included in the coliform count. Lactose-fermenting colonies must not be assumed to be *E. coli* and if required colony identification should be carried out.

Extension of the use of VRBA into food examination revealed weaknesses in using the ill-defined 'coli-aerogenes' group of organisms for assessments of processing and general hygiene. Lack of standardisation of methodology and differences in interpretation of colony morphology of coli-aerogenes organisms sometimes led to very considerable differences in accuracy and precision of results. This situation is brought about because generally all Gram-negative bacteria capable of growth on bile-containing media and which ferment lactose are included in the coliform count. This mixture of organisms may vary because of a number of influences including the type of sample under investigation, the culture medium used, incubation temperature and criteria chosen for reading the results. This last issue might erroneously exclude relevant organisms because of an unusual colony appearance; such as colour, size and presence or absence of bile precipitation surrounding colonies.

Substitution of glucose for lactose in the same selective medium resulted in Violet Red Bile Glucose Agar CM485. All Enterobacteriaceae on this medium produce 'purple-red' colonies and use of the clearly delineated family of Enterobacteriaceae eliminates the inaccuracies in methods and interpretation inherent in the ill-defined 'coli-aerogenes' group. However, there is the possibility of falsely reassuring results in situations where lactose-negative organisms predominate and where these may include non-lactose-fermenting pathogens, e.g. *Salmonella* species. In summary, generally little is to be gained by continued use of 'coli-aerogenes' bacteria instead of Enterobacteriaceae as index or indicator organisms but much may be lost. Violet Red Bile Glucose Agar is becoming the preferred medium for use in many investigations into raw materials, processed foods and plant hygiene. However, when testing, for example, raw vegetables, Violet Red Bile Agar may remain a more practical choice for the assessment of their hygienic status because certain non-lactose-fermenting but glucose-utilising organisms, e.g. *Pseudomonas* species predominate amongst the naturally-occurring associated flora and may easily overgrow the indicator organisms on VRBGA.

If there is any concern that non-lactose-fermenting pathogens may also be present, then consideration should be given to performing additional specific tests for these organisms, if necessary by a consulting laboratory.

Further information about the significance of Enterobacteriaceae present may be obtained by incubating VRBGA at different temperatures. When testing for enteric pathogens is not feasible, incubation at elevated temperatures, e.g. 42–44°C for detecting populations of thermotrophic Enterobacteriaceae may be helpful because these organisms and major enteric pathogens thrive at similar temperatures. **A test for thermotrophic Enterobacteriaceae should not be regarded as a substitute for a search for specific enteric pathogens.**

Some non-Enterobacteriaceae such as *Aeromonas* species may also grow on VRBA and VRBGA. It is important to have a general understanding of the microflora likely to be encountered in any specific sample. This will assist in the selection of the most appropriate medium and also guide the microbiologist in deciding the necessity for colony identification.

Because of the multiplicity of genera designated 'coli-aerogenes' and Enterobacteriaceae that grow on VRBA and VRBGA, variety may be expected in their colony appearance and size. These differences may be further influenced by parameters such as the numbers of colony-forming units present, their distance from each other and by incubation temperature. Although generally colonies may be up to 2mm in diameter, they may be considerably smaller, sometimes less than 0.5mm. In practice, all purple-red colonies on Violet Red Bile Agar CM107 should be regarded as presumptive coli-aerogenes and all purple-red colonies on Violet Red Bile Glucose Agar CM485 as Enterobacteriaceae. If required, further tests may be carried out to confirm their identity.

Mossel[1] has carried out much of the work on the role of Violet Red Bile Agar and Violet Red Bile Glucose Agar in food microbiology and his paper is a useful introduction to the subject. Further detailed discussion and methodology for examining foods for the presence of Enterobacteriaceae and coli-aerogenes (coliform) bacteria is given by Mossel et al[2].

Neither VRBA or VRBGA are intended to be used for detection of enteropathogenic *E. coli*. If it is necessary to determine whether any of the *E. coli* colonies present are of pathogenic strains then it will be necessary first to establish the serogroup and subsequently submit the isolate to tests of pathogenicity.

References
1 Mossel D.A.A. (1985) *Int. J. Food Microbiol.* 2. 27–32.
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VIOLET RED BILE LACTOSE AGAR

Code: CM107

A lactose-containing selective medium for the detection and enumeration of coliform organisms in water, food and dairy products.

Formula	gm/litre
Yeast extract	3.0
Peptone	7.0
Sodium chloride	5.0
Bile Salts No.3	1.5
Lactose	10.0
Neutral red	0.03
Crystal violet	0.002
Agar	12.0
pH 7.4 $\pm$ 0.2	

Directions
Suspend 38.5g in 1 litre of distilled water. Bring to the boil. Continue to boil for 2 minutes or for the minimum time necessary to dissolve completely and ensure that there are no remaining flecks of unmelted agar. No further sterilisation is necessary or desirable. Mix well before pouring.

Description
Violet Red Bile Lactose Agar is a selective medium for the detection and enumeration of coliform organisms. The medium has been used for the determination of the coli-aerogenes content of water, milk and other dairy products, dairy equipment, and food products etc[1,2].

Organisms which rapidly attack lactose produce purple colonies surrounded by purple haloes. Non-lactose or late-lactose fermenters produce pale colonies with greenish zones. Other related Gram-negative bacteria may grow but can be suppressed by incubation at >42°C or by anaerobic incubation.

Druce et al.[3] found that Violet Red Bile Lactose Agar was as good an indicator of coli-aerogenes bacteria in milk as MacConkey Broth, and that the Oxoid medium was suitable for determining the coli-aerogenes content of milk.

Technique
Druce et al. recommended the following procedures:

For the routine determination of the coli-aerogenes content of raw milk, prepare pour-plates containing 1.0, 0.1 and 0.01ml of the sample in Violet Red Bile Lactose Agar, and incubate for 20–24 hours at 35°C.

For coli-aerogenes counts of pasteurised milk, employ 4 pour-plates of Violet Red Bile Lactose Agar. Divide 10ml of the sample among three of the plates, and add 1ml of the sample to the remaining plate. Incubate for 20 to 24 hours at 30°C. Similarly the examination of rinses and swabs from dairy equipment and apparatus, should include the spreading of 10ml of solution on each of 3 plates and of 1ml on a single plate. Coliform organisms form dark red colonies which are 1 to 2mm in diameter, usually surrounded by a reddish zone. Occasionally colonies may be considerably smaller (less than 0.5mm in diameter).

When preparing pour-plates the medium should be freshly made up, cooled to 47°C and used within 3 hours.

An overlay method is helpful to improve the specificity of the medium. In this case a thin layer of cooled molten medium is poured over the inoculated base layer and allowed to set before incubation. Incubation may be carried out at >42°C for 18 hours, 32°C for 24–48 hours or 4°C for 10 days, depending on the temperature characteristics of the organisms to be recovered. For *Esch. coli* a temperature of 44° $\pm$ 1°C is specifically recommended[4].

Characteristic appearance of colonies
Round, purple-red< 0.5–2mm may be surrounded by purple-red haloes (lactose-positive organisms). Pale, may have greenish haloes (lactose-negative organisms).

Confirmation of the identity of red colonies must be made by further tests.

Storage conditions and Shelf Life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C and use as freshly as possible.

Quality Control
Positive control:
Escherichia coli ATCC® 25922

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions
This medium is not completely specific for Enterobacteriaceae, other organisms e.g. *Aeromonas* and *Yersinia* species may give similar reactions. The selectivity of the medium diminishes after 24 hours incubation and organisms previously suppressed may exhibit growth.

References
1 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.*
2 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.*
3 Druce R. G., Bebbington N. N., Elson K., Harcombe J. M. and Thomas S. B. (1957) *J. Appl. Bact. 20. 1–10.*
4 Mossel D. A. A. and Vega C. L. (1973) *Hlth Lab. Sci. 11. 303–307.*

VIOLET RED BILE GLUCOSE AGAR

Code: CM485

A glucose-containing selective medium for the detection and enumeration of Enterobacteriaceae in food products.

Formula	gm/litre
Yeast extract	3.0
Peptone	7.0
Sodium chloride	5.0
Bile Salts No.3	1.5
Glucose	10.0
Neutral red	0.03
Crystal violet	0.002
Agar	12.0
pH 7.4 $\pm$ 0.2	

Directions
Suspend 38.5g in 1 litre of distilled water. Bring to the boil. Continue to boil for 2 minutes or for the minimum time necessary to dissolve completely and ensure that there are no remaining flecks of unmelted agar. No further sterilisation is necessary or desirable. Mix well and dispense into tubes or dishes.

Description
Results from tests that may be applied to water to detect coli-aerogenes organisms as possible indicators of faecal contamination possess far less significance when applied to raw foods. In the examination of foodstuffs, detection of a more defined group of organisms, the Enterobacteriaceae, that ferment glucose to produce acid and/or gas has been recommended[1,2]. In addition to coliforms this group includes salmonellae and shigellae, which do not ferment lactose, and enterotoxicogenic *Esch. coli*. It also contains organisms, such as *Klebsiella* and *Citrobacter*, which are more resistant to heat than coliforms and are therefore better indicators of failure of processes that use minimal heat.

The difficulties of measuring the total Enterobacteriaceae content of foodstuffs have been studied by Mossel et al.[3], who showed that the addition of glucose to an existing medium for the detection of coliforms improves the performance. They added 10g per litre of glucose to crystal violet neutral red bile lactose agar (Violet Red Bile Agar CM107), and named the modified formulation MacConkey Glucose Agar.

Further work by Mossel et al.[4,5] showed that the lactose could be omitted resulting in the formulation of Violet Red Bile Glucose Agar CM485. The continued inclusion of lactose would not provide test results leading to more accurate identification. Exclusion of lactose renders the medium more economical to make as less weight is required per litre.

Media that contain bile salts have an intrinsic toxicity for Enterobacteriaceae, even for cells that have not been under stress[6,7,8,9,10,11].

Considerable differences have been observed among six commercial preparations of Violet Red Bile Agar[4,5] with regard to productivity for Enterobacteriaceae[12], and the intensity of their metabolism. In conjunction with Oxoid the components of the medium were examined and Mossel drafted a specification as follows:

1 Approved media have to be clear and yield colonies of satisfactory size. They have to give reproducible counts of typical colonies of Enterobacteriaceae.
2 When challenged for intrinsic toxicity by an anaerobic metabolic test[13] using a strain of *Yersinia enterocolitica* (Serotype 03) as a sensitive indicator, media must promote adequate growth, acid formation and, where required, adequate gas formation.
3 Media have to satisfy the confirmation rate of typical colonies, i.e. the number of colonies confirmed as Enterobacteriaceae divided by the number of colonies tested.

Violet Red Bile Glucose Agar CM485 has been developed to satisfy all of these criteria and complies with the recommendations of ISO[14].

Technique
Prepare a series of dilutions of the samples so that at least one will be included that will yield 100–200 colonies from a 1ml aliquot. Transfer 1ml aliquots of each dilution to 9cm petri dishes using 2 plates for each dilution. Add 15ml of medium, cool to 47°C. Gently swirl the plates 3 times clockwise and 3 times anti-clockwise. After the medium has solidified overlay with 10ml of the same medium and leave to solidify. Invert the dishes and incubate at >42°C for 18 hours, 32°C for 24–48 hours or 4°C for 10 days depending on the groups of Enterobacteriaceae to be recovered[15].

The agar overlay ensures anaerobic conditions which suppress the growth of non-fermentative Gram negative bacteria. It also encourages the fermentation of glucose which favours the formation of clearly visible purple colonies, surrounded by a purple halo.

Characteristic appearance of colonies
Round, purple 1–2mm diameter surrounded by purple haloes. Although colony size is generally 1–2mm, size can be affected by a number of influences and all red colonies should be counted. Confirmation of the identity of these colonies must be made by further tests.

Storage conditions and Shelf Life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C and use as freshly as possible.

Quality Control
Positive control:
Escherichia coli ATCC® 25922

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions
This medium is not completely specific for Enterobacteriaceae, other organisms may grow e.g. *Aeromonas* and *Yersinia* species.

The selective activity of this medium diminishes after 24 hours incubation and organisms previously suppressed may exhibit growth.

Medium for the poured plate procedure should be freshly prepared, cooled to 47°C and used within 3 hours.

References

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2 Mossel D. A. A. (1958) *Zbl. Bakt. I. Ref. 166. 421–432.*
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VOGEL-JOHNSON AGAR

Code: CM641

A selective medium for the isolation of Staphylococcus aureus from clinical specimens and food.

Formula	gm/litre
Tryptone	10.0
Yeast extract	5.0
Mannitol	10.0
Dipotassium phosphate	5.0
Lithium chloride	5.0
Glycine	10.0
Phenol red	0.025
Agar	16.0
pH 7.1 ± 0.2	

Directions
Suspend 61 grams in 1 litre of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add 5.7ml of sterile 3.5% potassium tellurite solution SR30 (equivalent to 20ml of 1% potassium tellurite).

Description
Vogel-Johnson Agar, by selecting and identifying coagulase positive and mannitol-fermenting strains, permits the early detection of *Staphylococcus aureus* from heavily contaminated foods and clinical specimens. It corresponds to the specification of the United States Pharmacopoeia[1] in terms of its formula.

Vogel and Johnson[2] modified the Tellurite Glycine Agar formula of Zebovitz et al.[3] by doubling the mannitol concentration to 1% (w/v) and adding Phenol Red as a pH indicator. The enhanced fermentation reaction which occurs as a result of the increase in mannitol content is clearly indicated by the development of yellow zones surrounding the colonies.

Staph. aureus is able to reduce tellurite to metallic tellurium resulting in growth as black colonies.

During the first 24 hours of incubation contaminating organisms are almost completely inhibited by tellurite, lithium chloride and the high glycine concentration. Virtually all the organisms that grow in this time are coagulase positive.

Organisms that grow as black colonies surrounded by a yellow zone after incubation at 35–37°C for 24 hours may be presumed to be *Staph. aureus*.

Prolonged incubation may result in the growth of black coagulase-negative colonies and if these organisms also ferment mannitol they may be falsely identified from their appearance as *Staph. aureus*. In these circumstances further tests of identity should be carried out before concluding that the organism is *Staph. aureus*.

Techniques

Food samples
1 Dry the surface of the plates.
2 With a glass spatula spread from 0.1 to 1.0ml of diluted food (macerated in 0.1% Peptone Water) over the surface of each well dried plate.
3 Incubate at 35–37°C and examine after 24 and 48 hours.

Clinical specimens
1 Dry the surface of the prepared plates.
2 Inoculate directly with the specimen.
3 Incubate at 35–37°C and examine after 24 and 48 hours.

Colonial appearance
Staphylococcus aureus appear as black, convex shiny colonies surrounded by a yellow zone.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Staphylococcus aureus ATCC® 25923

Negative control:
Escherichia coli ATCC® 25922

Precautions
All presumptive *Staph. aureus* colonies should be confirmed with further tests. Do not heat the medium after the addition of potassium tellurite.

References

1 United States Pharmacopoeia XXI (1985) *Microbial. Limit Tests. Rockville. Md.*
2 Vogel R. A. and Johnson M. J. (1961) *Pub. Hlth Lab. 18. 131.*
3 Zeboritz E., Evans J. B. and Niven C. F. (1955) *J. Bact. 70. 687.*

WILKINS-CHALGREN ANAEROBE AGAR

Code: CM619

A medium for the general growth of anaerobes, recommended for antimicrobial susceptibility testing. See Antimicrobial Susceptibility Testing Section for details of the use of this medium in AST methodology.

Formula	gm/litre
Tryptone	10.0
Gelatin peptone	10.0
Yeast extract	5.0
Glucose	1.0
Sodium chloride	5.0
L-Arginine	1.0
Sodium pyruvate	1.0
Menadione	0.0005
Haemin	0.005
Agar	10.0
pH 7.1 $\pm$ 0.2	

Directions
Suspend 43 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Recognising the need for a standard medium for antimicrobial susceptibility testing of anaerobic bacteria, Wilkins and Chalgren[1] developed a new medium which would not require the addition of blood. Their formulation included yeast extract to supply vitamins and other growth factors such as purines and pyrimidines, that are necessary for good growth of *Peptostreptococcus anaerobius* and *Bacteroides melaninogenicus*. Arginine was added to ensure sufficient amino acid was available for the growth of *Eubacterium lentum*. Pyruvate was added as an energy source, for asaccharolytic cocci such as *Veillonella*[2]. It also acts similarly to catalase and degrades traces of hydrogen peroxide, which may be produced by the action of molecular oxygen on medium constituents and interfere with the metabolism of anaerobes[3]. Haemin was found to be essential for the growth of *Bacteroides* species[4] and menadione for *B. melaninogenicus*[5].

Peptones derived from the single protein sources casein and gelatin, were used to improve standardisation of the medium. Wilkins and Chalgren[1] considered that this medium consistently grew anaerobes as well or better than media such as Brucella Agar or Schaedler Blood Agar. A collaborative study in ten laboratories showed that it could be used in an agar dilution method for susceptibility testing of anaerobic bacteria and recommended a procedure as a reference method[6].

The value of such a procedure was further confirmed by Brown and Waatti[7], who found that the incidence of resistance of anaerobic bacteria to frequently used antibiotics had increased. They considered it essential that diagnostic laboratories should have the capability of carrying out susceptibility tests on anaerobic bacteria.

Wilkins-Chalgren Agar has been recommended for the susceptibility testing of anaerobic bacteria using the Receiver Operating Characteristic (ROC) procedure.

Wilkins-Chalgren Anaerobe Agar is also recommended for the isolation of anaerobic organisms from clinical specimens. It has been shown to function well both in petri dishes and roll tubes. (B.S. Drasar, personal communication).

References

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2 Rogosa M. (1964) *J. Bacteriol. 87. 162–170.*
3 Hoffman P. S., George H. A., Kreig N. R. and Smibert R. A. (1979) *Can. J. Microbiol. 25. 8–16.*
4 Quinto G. and Sebald M. (1964) *Am. J. Med. Technol. 30. 381–384.*
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7 Brown W. J. and Waatti P. E. (1980) *Antimicrob. Agents Chemother. 17. 629–635.*
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N-S ANAEROBE SELECTIVE SUPPLEMENT

Code: SR107

For the selective isolation of non-sporing anaerobes.

Vial contents (each vial is sufficient for 500ml of medium)

Haemin	2.5mg
Menadione	0.25mg
Sodium pyruvate	500mg
Nalidixic acid	5mg

G-N ANAEROBE SELECTIVE SUPPLEMENT

Code: SR108

For the selective isolation of Gram-negative anaerobes.

Vial contents (each vial is sufficient for 500ml of medium)

Haemin	2.5mg
Menadione	0.25mg
Sodium succinate	1,250mg
Nalidixic acid	5.0mg
Vancomycin	1.25mg

Directions

To prepare Non-Selective Medium for all anaerobic organisms
Suspend 21.5g of Wilkins-Chalgren Anaerobe Agar CM619 in 475ml of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add 25ml defibrinated blood SR50/SR51. Mix gently, and pour into sterile petri dishes (see plate 1).

To prepare Selective Medium for Non-Sporing Anaerobes
Suspend 21.5g of Wilkins-Chalgren Anaerobe Agar CM619 in 475ml of distilled water containing 0.5ml

'Tween 80'. Bring to the boil to dissolve completely and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50–55°C and aseptically add the contents of 1 vial of N-S Anaerobe Supplement SR107 rehydrated with 10ml of sterile distilled water, together with 25ml of defibrinated blood SR50/SR51. Mix gently and pour into sterile petri dishes (see plate 2).

To prepare Selective Medium for Gram-Negative Anaerobes
Suspend 21.5g of Wilkins-Chalgren Anaerobe Agar CM619 in 475ml of distilled water. Bring to the boil to dissolve completely and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50–55°C and aseptically add the contents of 1 vial of G-N Anaerobe Supplement SR108 rehydrated with 10ml of sterile distilled water, together with 25ml defibrinated blood SR50/SR51. Mix gently and pour into sterile petri dishes (see plate 3).

Wilkins-Chalgren Anaerobe Agar is recommended but other media may be used satisfactorily, e.g. Columbia Agar Base CM331 and Blood Agar Base No.2 CM271. Sufficient haemin and menadione are contained in N-S and G-N Supplements to provide adequate levels in these media when used as directed.

Note
Use N-S Supplement with Wilkins-Chalgren Medium for NAT medium. Use G-N Supplement with Wilkins-Chalgren Medium for NAV Medium[1].

Discussion
Selective Medium for Non-Sporing Anaerobes
This medium is referred to in the published literature[1] as NAT Medium and is recommended for the isolation of non-sporing anaerobes from clinical specimens.

The recovery of non-sporing anaerobes from clinical material may sometimes prove difficult in specimens containing mixtures of aerobic and anaerobic bacteria. A medium which contains nalidixic acid as the selective agent was described by Wren[1] for isolating these organisms. It was shown to be virtually non-inhibitory to most non-sporing anaerobes whilst retaining good selectivity for these organisms when present in mixed cultures. The medium is particularly useful for the recovery of non-sporing Gram-positive anaerobes since the presence of 'Tween 80' stimulates their growth[2].

Another advantage of this medium is the earlier colonial pigmentation of the *Bacteroides melaninogenicus* group due to the slow lysis of the blood by 'Tween 80' during incubation. It is also a less inhibitory medium than aminoglycoside-containing media for non-sporing anaerobes in general.

The NS Anaerobe Supplement SR107 for non-sporing anaerobes contains nalidixic acid as the selective agent, together with haemin, menadione and sodium pyruvate as an additional energy source[1,4].

Haemin was found to be essential for the growth of *Bacteroides* species[5] and menadione for *B. melaninogenicus*.[6] Pyruvate, in addition to being an energy source, acts similarly to catalase and degrades traces of hydrogen peroxide which may be produced by the action of molecular oxygen on media constituents. Hydrogen peroxide is known to affect the metabolism of anaerobes[7].

Downes et al.[8] showed that NAT Medium was superior to kanamycin agar (KA) and neomycin agar (NA) in the recovery of all non-clostridial anaerobes. The major superiority was in the recovery of anaerobic, Gram-positive cocci.

Selective Medium for Gram-Negative Anaerobes
This medium is described[9] as NAV Medium and is recommended for the isolation of Gram-negative anaerobes from clinical specimens.

NAV Medium is a modification of NAT Medium[1] in which 'Tween 80' and sodium pyruvate have been replaced by sodium succinate. Vancomycin has been added, thus making the medium totally selective for Gram-negative anaerobes.

G-N Anaerobe Supplement SR108 contains nalidixic acid and vancomycin as selective agents; haemin, menadione and sodium succinate as growth factors. Haemin was found to be essential for the growth of *Bacteroides* species[5] and menadione for *B. melaninogenicus*[6]. Some Gram-negative anaerobes require succinate as a source of energy[10].

The recovery of Gram-negative anaerobes on NAV Medium has been shown[8] to be superior to that on media containing neomycin and kanamycin as selective agents.

In order to isolate the maximum non-sporing anaerobic bacteria from clinical specimens the following scheme must be followed.

Specimen
Inoculate on to each of the following media and incubate anaerobically for 48 hours.

Plate 1	***Plate 2***	***Plate 3***
Wilkins-Chalgren Anaerobe Agar CM619 + 5% (v/v) defibrinated blood	CM619 + 'Tween 80' +5%(v/v) defibrinated blood + SR107 (NAT Medium)	CM619 + 5% (v/v) defibrinated blood + SR108 (NAV Medium)
All bacteria capable of growing under anaerobic conditions	Non-Sporing Gram +ve and Gram -ve anaerobic bacteria	Gram-negative anaerobic bacteria

A non-selective plate is included for attempted isolation of any strain, in particular *B. corrodens* which is sensitive to the selective agents.

Technique
1 Prepare supplies of Plate 1 (CM619 + blood) Plate 2 (CM619 + blood + Tween 80 + SR107) and Plate 3 (CM619 + blood + SR108) as described in the section marked Directions.
2 Inoculate the specimens on to plates of each medium. Best results are obtained if freshly prepared plates are used but plates may be stored at 4°C for up to 3 days.
3 Incubate the plates anaerobically at 35°C for 48 hours. The Oxoid Anaerobic System with a Gas Generating Kit BR38 is recommended. Alternatively use Anaerogen AN025A or AN035A.

Anaerogen does not require the addition of water or a catalyst.

4 Examine the plates. If no growth has occurred then incubation should be continued up to 5 days before plates are discarded, as up to 20% of non-sporing anaerobes require prolonged incubation under unbroken anaerobic conditions.

5 Carry out confirmatory tests on the isolates and record the results as follows:
   (i) all facultative anaerobes and obligate anaerobes isolated on the Wilkins-Chalgren Anaerobe Agar Medium plate.
   (ii) all non-sporing anaerobes isolated on the medium for non-sporing anaerobes.
   (iii) all Gram-negative anaerobes isolated on the medium for Gram-negative anaerobes.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates of medium at 2–8°C away from light.

Quality Control
Positive control:
Clostridium perfringens ATCC® 13124
Bacteroides fragilis ATCC® 25285

Negative control:
Uninoculated medium

NAT Medium modification
Positive control:
Prevotella loescheii ATCC® 15930
Peptococcus magnus ATCC® 14956

Negative control:
Escherichia coli ATCC® 25922

NAV Medium modification
Positive control:
Bacteroides fragilis ATCC® 25285
Fusobacterium necrophorum ATCC® 25286

Negative control:
Escherichia coli ATCC® 25922

References

1 Wren M. W. D. (1977) *J. Med. Microbiol. 10. 195–201.*
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4 Rogosa M. (1964) *J. Bacteriol. 87. 162–170.*
5 Quinto G. and Sebald M. (1964) *Am. J. Med. Technol. 30. 381–384.*
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WILKINS-CHALGREN ANAEROBE BROTH

Code: CM643

A medium for the general growth and antimicrobial susceptibility testing of anaerobic organisms. See Antimicrobial Susceptibility Testing Section 6 for details of the use of this medium in AST methodology.

Formula	gm/litre
Tryptone	10.0
Gelatin peptone	10.0
Yeast extract	5.0
Glucose	1.0
Sodium chloride	5.0
L-Arginine	1.0
Sodium pyruvate	1.0
Menadione	0.0005
Haemin	0.005
pH 7.1 $\pm$ 0.2	

Directions
Suspend 33 grams in 1 litre of warm distilled water. Mix well, distribute into final containers and sterilise by autoclaving at 121°C for 15 minutes.

Description
Wilkins-Chalgren Anaerobe Broth CM643 is derived from Wilkins-Chalgren Anaerobe Agar[1] CM619.

Where studies on antimicrobial susceptibilities are being made both in broth and agar, standardisation is improved by using media of identical nutrient formulation.

The growth of anaerobic organisms in this broth is particularly good. The formulation includes yeast extract to supply vitamins and other growth factors such as purines and pyrimidines, that are necessary for good growth of *Peptostreptococcus anaerobius* and *Bacteroides melaninogenicus*. Arginine is added to ensure sufficient amino-acid is available for the growth of *Eubacterium lentum*. Pyruvate is present as an energy source for asaccharolytic cocci such as *Veillonella*. It also acts similarly to catalase and degrades traces of hydrogen peroxide, which may be produced by the action of molecular oxygen on medium constituents and interfere with the metabolism of anaerobes. Haemin is found to be essential for the growth of *Bacteroides* species and menadione for *B. melaninogenicus*.

Peptones derived from the single protein sources casein and gelatin are used to improve standardisation of the medium. The early heavy growth that is usual may reflect the absence of Eh-reducing substances that can be inhibitory to some organisms.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C away from light.

Quality Control
Positive control:
Peptostreptococcus anaerobius ATCC® 27337
Prevotella loescheii ATCC® 15930

Negative control:
Uninoculated medium

Reference

1 Wilkins T. D. and Chalgren S. (1976) *Antimicrob. Agents Chemother. 10. 926–928.*

WL NUTRIENT AGAR

Code: CM309

A medium for the determination of the microbiological flora in brewing and fermentation which can be made selective for bacteria with cycloheximide.

Formula	gm/litre
Yeast extract	4.0
Tryptone	5.0
Glucose	50.0
Potassium dihydrogen phosphate	0.55
Potassium chloride	0.425
Calcium chloride	0.125
Magnesium sulphate	0.125
Ferric chloride	0.0025
Manganese sulphate	0.0025
Bromocresol green	0.022
Agar	15.0
pH 5.5 $\pm$ 0.2	

Directions

Suspend 75 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. If required the pH may be adjusted to 6.5 by the addition of 1% sodium bicarbonate solution.

Description

WL Nutrient Medium, based on that of Green and Gray[1] is recommended for the determination of the microbiological flora in brewing and fermentation processes. The medium is suitable for the differentiation of 'wild' yeasts from brewing yeasts[2].

Reliable counts for brewers' yeast are obtained with the medium at pH 5.5. Adjustment to pH 6.5 facilitates the counting of bakers' and distillers' yeasts.

With the addition of cycloheximide 0.004gm/litre, to suppress yeast growth, the medium becomes selective for the bacterial contaminants of yeast cultures.

When making microbial counts with this medium the time and temperature of incubation will vary according to the materials tested and the organisms sought. Temperatures of 25°C are used for brewing materials and 30°C for bakers' yeasts.

Incubation

Times can vary from 2 to 14 days. Aerobic or anaerobic incubation conditions will depend on the characteristics of the organisms.

Storage conditions and Shelf life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control

w/o cycloheximide

Positive control:
Saccharomyces cerevisiae ATCC® 9763

Negative control:
Uninoculated medium

with cycloheximide

Positive control:
Escherichia coli ATCC® 25922

Negative control:
S. cerevisiae ATCC® 9763

Precautions

When handling cycloheximide observe the precautions to be taken under HAZARDS page 2–7.

References

1 Green S. R. and Gray P. P. (1950) *Wallerstein Lab. Comm. 13. 357.*
2 Hall Jean F. (1971) *J. Inst. Brewing 77. 513–516.*

WL NUTRIENT BROTH

Code: CM501

A liquid medium for use in the control of brewing and other fermentation processes.

Formula	gm/litre
Yeast extract	4.0
Tryptone	5.0
Glucose	50.0
Potassium dihydrogen phosphate	0.55
Potassium chloride	0.425
Calcium chloride	0.125
Magnesium sulphate	0.125
Ferric chloride	0.0025
Manganese sulphate	0.0025
Bromocresol green	0.022
pH 5.5 $\pm$ 0.2	

Directions

Dissolve 60g in 1 litre of distilled water. Mix well and distribute into containers. Sterilise by autoclaving at 121°C for 15 minutes.

The pH may be raised to 6.5 by the addition of 1% w/v sodium bicarbonate solution. Adding 0.004 grams of cycloheximide per litre of broth will form WL Differential Broth. Note the precautions to be taken under HAZARDS page 2–7.

Description

WL Nutrient Broth is based on the formulation of Green and Gray[1] and is used where there are advantages for broth media e.g. using larger samples of liquid products or for enrichment cultures with cycloheximide.

Addition of 0.004 grams/litre of cycloheximide suppresses yeast growth and renders the medium selective for bacterial contaminants. Adjustment of the medium to pH 6.5 facilitates growth of bakers' and distillers' yeasts; the medium at pH 5.5 is used for growth of bakers' yeasts.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
w/o cycloheximide
Positive control:
Saccharomyces cerevisiae ATCC® 9763

Negative control:
Uninoculated medium

with cycloheximide
Positive control:
Escherichia coli ATCC® 25922

Negative control:
S. cerevisiae ATCC® 9763

Precautions
When handling cycloheximide observe the precautions to be taken under HAZARDS page 2–7.

References
1 Green S. R. and Gray P. P. (1950) *Wallerstein Lab. Comm. 13. 357.*
2 Hall Jean F. (1971) *J. Inst. Brewing 77. 513–516.*

WORT AGAR

Code: CM247

A medium for the cultivation and enumeration of yeasts.

Formula	gm/litre
Malt extract	15.0
Peptone	0.78
Maltose	12.75
Dextrin	2.75
Glycerol	2.35
Dipotassium phosphate	1.0
Ammonium chloride	1.0
Agar	15.0
pH 4.8 ± 0.2	

Directions
Suspend 50g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

PROLONGED OR EXCESSIVE HEATING WILL DIMINISH THE GEL STRENGTH OF THE AGAR.

Description
Wort Agar is a general purpose mycological medium, equivalent to the medium described by Parfitt[1] and especially suitable for the cultivation and enumeration of yeasts. The medium which duplicates the composition of natural wort, is of an acidity which is optimal for many yeasts but inhibitory to most bacteria. Parfitt investigated the relative merits of wort agar and other media for the count of yeasts and moulds in butter, and recommended the use of dehydrated whey, malt or wort agar for the purpose. Scarr[2] employed a modified wort agar ('osmophilic agar') for the examination of sugar products for osmophilic yeasts. Scarr's technique is also used for the determination of osmophilic yeasts occurring in materials used in the manufacture of soft drinks.

Technique
For the microbiological examination of butter, make suitable dilutions in quarter-strength Ringer solution (prepared with Ringer Solution Tablets BR52). Transfer 1ml of each dilution to a separate petri dish; add 15ml of melted Wort Agar, cooled to 45°C to 48°C; mix by rotary movements in a horizontal plane; allow the poured-plates to set (protected from the light) at room temperature for 30–50 minutes. Incubate in an inverted position, e.g. for 5 days at 25°C, and count the number of yeasts and mould colonies which develop.

For the examination of sugar products for osmophilic yeasts, dissolve dehydrated Wort Agar in a syrup containing 35 parts w/w of sucrose and 10 parts w/w of glucose, and autoclave for 20 minutes at 110°C. Inoculate and mix as above. Scarr recommends incubation at 27°c for 3–4 days for *Schizosaccharomyces* species and for 5 days for less common osmophilic yeasts. Colonies on the medium are well defined and normally opaque.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Saccharomyces cerevisiae ATCC® 9763

Negative control:
Escherichia coli ATCC® 25922

Precautions
Do not remelt the solid agar, it will destroy the gel. The surface of the agar is soft but suitable for poured inocula.

References
1 Parfitt E. H. (1933) *J. Dairy Sci. 16. 141–147.*
2 Scarr M. Pamela (1959) *J. Sci. Food Agric. 10(12). 678–681.*

XLD MEDIUM

Code: CM469

A selective medium for the isolation of salmonellae and shigellae from clinical specimens and foods.

Formula	gm/litre
Yeast extract	3.0
L-Lysine HCl	5.0
Xylose	3.75
Lactose	7.5
Sucrose	7.5
Sodium desoxycholate	1.0
Sodium chloride	5.0
Sodium thiosulphate	6.8
Ferric ammonium citrate	0.8
Phenol red	0.08
Agar	12.5
pH 7.4 $\pm$ 0.2	

Directions
Suspend 53g in 1 litre of distilled water. Heat with frequent agitation until the medium boils. DO NOT OVERHEAT. Transfer immediately to a water bath at 50°C. Pour into plates as soon as the medium has cooled.

It is important to avoid preparing large volumes which will cause prolonged heating.

Description
Xylose-Lysine-Desoxycholate Agar was originally formulated by Taylor[1] for the isolation and identification of shigellae from stool specimens. It has since been found to be a satisfactory medium for the isolation and presumptive identification of both salmonellae and shigellae[2]. It relies on xylose fermentation, lysine decarboxylation and production of hydrogen sulphide for the primary differentiation of shigellae and salmonellae from non-pathogenic bacteria.

Rapid xylose fermentation is almost universal amongst enteric bacteria, except for members of the *Shigella, Providencia* and *Edwardsiella* genera[1]. Xylose is thus included in the medium so that *Shigella* spp. may be identified by a negative reaction.

Salmonella spp. are differentiated from non-pathogenic xylose fermenters by the incorporation of lysine in the medium. Salmonellae exhaust the xylose and decarboxylate the lysine, thus altering the pH to alkaline and mimicking the *Shigella* reaction. However, the presence of *Salmonella* and *Edwardsiella* spp. is differentiated from that of shigellae by a hydrogen sulphide indicator.

The high acid level produced by fermentation of lactose and sucrose, prevents lysine-positive coliforms from reverting the pH to an alkaline value, and non-pathogenic hydrogen sulphide producers do not decarboxylate lysine. The acid level also prevents blackening by these micro-organisms until after the 18 to 24 hour examination for pathogens.

Sodium desoxycholate is incorporated as an inhibitor in the medium. The concentration used allows for the inhibition of coliforms without decreasing the ability to support shigellae and salmonellae.

The recovery of *Shigella* spp. has previously been neglected despite the high incidence of shigellosis. This has been due to inadequate isolation media[3]. The sensitivity and selectivity of XLD Agar exceeds that of the traditional plating media e.g. Eosin Methylene Blue, Salmonella-Shigella, and Bismuth Sulphite agars, which tend to suppress the growth of shigellae. Many favourable comparisons between XLD Agar and these other media have been recorded in the literature[4,2,5,6,7,8,9,10].

The recovery of salmonellae and shigellae is not obscured by profuse growth of other species[3] therefore XLD Agar is ideal for the screening of samples containing mixed flora and suspected of harbouring enteric pathogens e.g. medical specimens or food products. Chadwick, Delisle and Byer[11] recommended the use of this medium as a diagnostic aid in the identification of Enterobacteriaceae.

XLD Agar, in conjunction with MLCB Agar, is specified for use following enrichment culture in Modified Semi-Solid Rappaport Medium (MSRV) when examining faeces for *Salmonella* spp[12].

Technique
Faeces or rectal swabs may be plated directly[13] or selective enrichment broths may be used prior to streaking out. Selenite Broth CM395 or Tetrathionate Broth CM29 may be used for salmonella enrichment.

1 Inoculate the poured, dried plates with a loopful of inoculum either from a suitable enrichment broth, from stool samples or rectal swabs.
2 Incubate the plates at 35–37°C for 18 to 24 hours.

Colonial Appearances

Organism	***Appearance***
Salmonella Edwardsiella	Red colonies with black centres
Shigella Providencia H_2S-negative Salmonella (e.g. *S. paratyphi A*)	Red colonies
Escherichia *Enterobacter* *Klebsiella* *Citrobacter* *Proteus* *Serratia*	Yellow, opaque colonies

Note
False positive, red colonies may occur with some *Proteus* and *Pseudomonas* species.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922

References

1 Taylor W.I. (1965) *Am. J. Clin. Path. 44. 471–475.*
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3 Isenberg H.D., Kominos S. and Sigeal M. (1969) *Appl. Microbiol. 18. 656–659.*
4 Taylor W. I. and Harris B. (1965) *Am. J. Clin. Path. 44. 476–479.*
5 Taylor W. I. and Harris B. (1967) *Am. J. Clin. Path. 48. 350–355.*
6 Taylor W. I. and Schelhart D. (1967) *Am. J. Clin. Path. 48. 356–362.*
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8 Rollender M.A., Beckford O., Belsky R.D. and Kostroff B. (1969) *Am. J. Clin. Path. 51. 284–286.*
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10 Dunn C. and Martin W.J. (1971) *Appl. Microbiol. 22. 17–22.*
11 Chadwick P., Delisle G.H. and Byer M. (1974) *Can. J. Microbiol. 20. 1653–1664.*
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YEAST EXTRACT AGAR

Code: CM19

A nutrient agar for the plate count of organisms in water.

Formula	gm/litre
Yeast extract	3.0
Peptone	5.0
Agar	15.0
pH 7.2 ± 0.2	

Directions
Suspend 23g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description
This medium is made to the formula described by Windle Taylor for the plate count of micro-organisms in water[1].

In the UK the usual method of counting heterotrophic bacteria in water is by the poured plate method with Yeast Extract Agar. Seperate counts are made of those aerobic mesophilic organisms which form visible colonies in this medium after 24 hours incubation at 35°C and those which form colonies after 3 days at 20–22°C. The two methods give different results[2].

The organisms growing under these conditions comprise bacteria, yeasts and moulds.

Technique
1 Prepare appropriate decimal dilutions of the water sample (with Ringer Solution CM52) and pipette 1ml portions of the water and each dilution into duplicate sterile petri dishes.
2 Add 15ml of Yeast Extract Agar (previously melted and cooled to 45–50°C) to each plate. Mix the contents by a combination of rapid to-and-fro shaking and circular movements lasting over a period of 5–10 seconds.
3 Allow to solidify, and incubate duplicate sets of plates for 24 hours at 37°C and 3 days at 20–22°C respectively.
4 Select plates containing 30–300 colonies for counting. No count should be made on a plate containing less than 30 colonies unless the plates from the undiluted water contain less than this number.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control
Positive control:
Escherichia coli ATCC® 25922

Negative control:
Uninoculated medium

References

1 Windle Taylor E. (1958) *'The Examination of Waters and Water Supplies', 7th ed., Churchill Ltd., London, pp. 394–398 and 778.*
2 DHSS. Report 71 (1982) *'The Bacteriological Examination of Drinking Water Supplies' HMSO. London. pp. 54–55.*

YEAST AND MOULD AGAR

Code: CM920

A medium for the isolation of yeasts and moulds.

Formula	gm/litre
Yeast extract	3.0
Malt extract	3.0
Peptone	5.0
Dextrose	10.0
Agar	20.0
Final pH 6.2 ± 0.2	

Directions
Suspend 41.0 grams of Yeast and Mould Agar CM920 in 1 litre of distilled water and bring to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. The medium may be rendered selective after sterilisation by acidifying to pH 4.0 with 12–15mls of Sterile Lactic Acid (Oxoid SR21) after cooling to 50°C. Do not reheat after making this addition. Mix well and pour into sterile petri dishes.

Description
Yeast and Mould Agar is based on the formulation described by Wickerham[1,2]. The medium is recommended for the isolation and maintenance of yeasts and moulds.

Yeast and Mould Agar may be rendered selective by the addition of acid to reduce the pH of the medium to pH 4.0. A suitable acid for use is Oxoid Sterile Lactic Acid (SR21).

Technique
1 Prepare Yeast and Mould Agar plates as directed in the directions for use.
2 Inoculate the medium by surface or poured plate procedures.
3 Incubate the plates for 48–72 hours at 25°C to 30°C.

Detection and enumeration of yeasts in the presence of moulds may be made easier by using a combined anaerobic/aerobic incubation procedure[3].

Reaction after incubation

		pH 6.2	pH 4.0
Aspergillus niger	ATCC® 16404	Good growth	Good growth
Candida albicans	ATCC® 10231	Good growth	Good growth
Saccharomyces serevisiae	ATCC® 9763	Good growth	Good growth
Lactobacillus fermentum	ATCC® 9338	Good growth	Partial to complete inhibition
Escherichia coli	ATCC® 25922	Good growth	Partial to complete inhibition

Cultures are initially incubated for 3 days under anaerobic conditions and then for a further 2 days aerobically. Development of mould colonies is impeded during the anaerobic phase of incubation. Dimorphic moulds e.g. (*mucor* spp) may form yeast-like colonies during anaerobic incubation.

Technique
Cultures on plates or membrane filters are incubated for 3 days at 25°C under strictly anaerobic conditions. Continue incubation of the cultures under aerobic conditions for a further 2 days. Yeast colonies may be very small immediately following anaerobic incubation but will increase in size in air. Mould growth may become completely unrestricted after 3 days in air.

Storage conditions and Shelf life
Yeast and Mould Agar should be stored tightly capped in the original container at 10°C to 25°C. When stored as directed the medium will remain stable until the expiry date printed on the label.

The prepared medium may be stored for up to 2 weeks at 2–8°C.

Quality Control
Yeast and Mould Agar may be tested for performance using stable, typical control cultures.

Precautions
For *in vitro* diagnostic use.

Do not use beyond the stated expiry date or if the product is caked, discoloured or shows any sign of deterioration.

References

1 Wickerham L.J. (1951) *U.S. Dept. Agric. Tech. Bull. No 1029, 1–19.*
2 Wickerham L.J. and Rettger L.F (1939) *J. Tropical Med. Hyg. 42. 174–179.*
3 De Jong J. and Put H.M.C. (1980) *Biology and Activities of Yeasts. Society for Applied Bacteriology Symposium series No. 9. Skinner F.A., Passmore S.M. and Davenport R.R. (eds). Academic Press, London. Pages 289–292.*

YERSINIA SELECTIVE AGAR BASE

Code: CM653

A selective medium for Yersinia enterocolitica when used with Yersinia Selective Supplement SR109 (Schiemann CIN Medium).

Formula	gm/litre
Special peptone	20.0
Yeast extract	2.0
Mannitol	20.0
Sodium pyruvate	2.0
Sodium chloride	1.0
Magnesium sulphate	0.01
Sodium desoxycholate	0.5
Neutral red	0.03
Crystal violet	0.001
Agar	12.5
pH 7.4 $\pm$ 0.2	

YERSINIA SELECTIVE SUPPLEMENT

Code: SR109

Vial contents (each vial is sufficient for 500ml of medium).

Cefsulodin	7.5mg
Irgasan	2.0mg
Novobiocin	1.25mg

Reconstitute the antibiotic supplement SR109 by aseptically adding 2ml of a sterile 50:50 solution of ethanol and water to one vial and mix gently to dissolve the contents completely.

Directions
Suspend 29.0g in 500ml of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Allow to cool to 50°C and aseptically add the contents of one vial of Yersinia Selective Supplement SR109 reconstituted as directed. Mix gently and pour into sterile petri dishes.

Description
Yersinia Selective Medium (CIN Medium) is based on the formulation of Schiemann[1,2] and is recommended for the isolation and enumeration of *Yersinia enterocolitica* from clinical specimens and food.

Y. enterocolitica is becoming increasingly recognised as a cause of diarrhoeal disease of man. Infection by the organisms results in diarrhoea, malaise, nausea and fever, plus constant abdominal pain over a period of 1–2 days. The organism has also been shown as a cause of polyarthritis, mesenteric adenitis and septicaemia. It is likely that human infections are directly or indirectly derived from animal sources and may be contracted through the ingestion of contaminated food. Initially serotypes 0:3 and 0:9 were implicated in human infections but since then other serotypes, mainly 0:5 and 0:8 have also been involved[3]. It is important to note that incidence of disease caused by the various serotypes of *Y. enteroccolitica* is currently reported to vary considerably with geographical location. It is expected that with provision of a selective medium, a higher isolation rate will result, and *Y. enterocolitica* will be recognised as more common and widespread than previously suspected.

Yersinia Selective Agar Base CM653 and the selective supplement SR109 have been developed specifically for the optimum growth and recovery of *Y. enterocolitica* after 18–24 hours incubation at 32°C. Schiemann[2] modified his earlier formulation for CIN Medium by replacing Bile Salts with sodium desoxycholate (0.5g/l) and by reducing the concentration of novobiocin from 15 to 2.5mg/l in order to eliminate the inhibition of some strains of serotype 0:8.

The typical colonies of *Y. enterocolitica* will develop as a dark red 'bullseye' surrounded by a transparent border and will vary considerably among serotypes in colony size, smoothness and the ratio of the border to centre diameter. Most other organisms that are capable of growing will produce larger colonies (>2mm in diameter) with diffuse pinkish centres and opaque outer zones. *Serratia liquefaciens, Citrobacter*

freundii and *Enterobacter agglomerans* may give a colonial morphology resembling *Y. enterocolitica*. These organisms can be differentiated from *Y. enterocolitica* by biochemical tests.

Test for growth on Nutrient and MacConkey Agars, test for indole and urease production and for acid reactions from sucrose, cellobiose, amygdalin, melibiose, rhamnose and reffinose. Carry out tests at 30°C rather than 37°C[4,5].

Technique for Culture

Direct Plate Method

1 Pour plates of Yersinia Selective Agar and dry the surface.
2 Inoculate the plates with a suspension of the food, faeces, etc., to produce single colonies.
3 Incubate at 32°C for 24 hours.

Cold Enrichment in Phosphate Buffered Saline[6]

1 Inoculate food, faeces, etc., into M/15 phosphate buffered saline.*
2 Hold at 4°C for up to 21 days.
3 Periodically sub-culture samples on to plates of Yersinia Selective Agar.
4 Incubate at 32°C for 24 hours.

*To prepare an M/15 buffer dissolve one tablet of Oxoid Dulbecco 'A' BR14A in 223ml of distilled water. Distribute into final containers and sterilise by autoclaving at 115°C for 10 minutes.

CIN Agar had been used for isolation of *Leptospira* spp[7]. With enhancement of its nutritional properties and addition of 5-fluorouracil to increase selectivity it has also been used to demonstrate the presence of *Arcobacter* spp. in ground pork[8].

Colonial Morphology

The typical colonies of *Y. enterocolitica* will develop a dark red 'bullseye' surrounded by a transparent border. The colony size, smoothness and the ratio of the border to centre diameter will vary considerably among serotypes.

Identification of Isolates

The presumptive colonies are confirmed as *Y. enterocolitica* by the biochemical reactions shown in Table 1.

Storage conditions and Shelf life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C for not more than 24 hours.

Quality Control

Positive control:
Yersinia enterocolitica ATCC® 27729

Negative control:
Escherichia coli ATCC® 25922

Precautions

Some strains of *Y. enterocolitica* may grow poorly or not at all. Other species of *Yersinia* may grow along with some enteric organisms. It is therefore essential that full identification tests are carried out on suspect colonies.

References

1 Schiemann D. A. (1979) *Can. J. Microbiol. 25. 1298–1304.*
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5 Mair N. S. and Fox E. (1986) *Yersiniosis: Laboratory Diagnosis, Clinical Features and Epidemiology. Pub. Hlth Lab. Ser. London.*
6 Pai C. H., Sorger S., Lafleur L., Lackman L. and Marks M. I. (1979) *J. Clin. Microbiol. 9. 712–715.*
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Table 1

Yersinia enterocolitica

Growth at 4°C and on Nutrient/MacConkey Agars
Motile at 22°C
Indole production variable
Urease positive
Ornithine decarboxylase positive
Acid production from sucrose, cellobiose, amygdalin, rhamnose and raffinose
No acid produced from melibiose.

QUALITY CONTROL ORGANISMS

OXOID CULTI-LOOPS®

These enable standardised cultures for quality control testing to be prepared quickly, easily and safely.

Culti-Loops are ideal for:

Performance testing of culture media, stains and diagnostic reagents

Evaluation of bacteriological procedures

Maintenance of stock cultures

Culti-Loops are ready to use, disposable bacteriological loops containing stabilised viable micro-organisms.

Culti-Loops are recommended for use in the performance testing of culture media, stains, diagnostic kits and reagents, for the maintenance of stock cultures and in the evaluation of bacteriological procedures. Each loop is individually packaged in a foil pouch and each can contains ten such loops.

Precautions
Culti-Loops contain viable micro-organisms and should be used only by individuals with bacteriological training. Refer to national Guidelines for Microbiological Containment Category information. After use, all loops and packaging should be placed into an appropriate container and sterilised by autoclaving before their final disposal. Do not place the loops into bunsen burners.

Storage
Store Culti-Loops at 2–8°C (or frozen for *Campylobacter* spp.). Remove only the quantity of loops required for immediate use. Under these conditions, Culti-Loops will retain their viability until the date shown on the foil pouch.

To open
Cut open the end of the foil packet as indicated on the label.

Evidence of deterioration
Each loop should contain an intact dried film. Do not use the loop if there is any evidence of hydration.

Procedure
The film in each loop is made from a gelatin formulation and then dried by special processing. To rehydrate the film, the loops must come into contact with both warmth and moisture.

Direct inoculation of Culti-Loops onto selective media may result in slow or absent growth. It is therefore recommended that where this is observed, inoculation onto non-selective media (such as blood agar) should precede sub-culture onto selective media.

The following two methods may be used for inoculation. Utilise the appropriate method for the selected micro-organism.

Direct Streak Method
This procedure is recommended for all non-fastidious micro-organisms.

1 Warm the appropriate plate medium to 37°C.
2 Remove the sheath from the loop.
3 Stab the loop into the medium or lay it flat on the warm, moist surface. Hold it in this manner for 10–15 seconds to allow for absorption of moisture.
4 Streak the plate in the usual manner. As many as five plates may be streaked with the same loop.
5 Incubate the plates in an appropriate atmosphere and temperature for the optimal growth of the organism.

Indirect (Broth) Method
This procedure is recommended for all fastidious micro-organisms.

1 Remove the sheath from the loop.
2 Cut off the loop shaft from the handle using sterilised scissors into a tube containing 0.5 to 1.0ml of liquid medium. Use:
   a Tryptone Soya Broth (Oxoid CM129) or freshly prepared Thioglycollate USP (CM391) for bacteria specimens.
   b Sterile saline for mycology specimens.
3 Place tube in a 37°C incubator just long enough for the film to dissolve completely out of the loop. Shake the tube gently to suspend the organism.
4 Using the Pasteur pipette, inoculate the appropriate media with several drops and streak in the usual manner.
5 Incubate the plates in an appropriate atmosphere and temperature for the optimal growth of the organism.

Most organisms grow in 24–48 hours under the proper conditions. However, some exhibit a considerable lag phase and should be incubated for an additional 24 hours.

Disclaimer
Those who receive Culti-Loops are responsible for their safe storage, handling and use. Oxoid is not liable for damages or injuries resulting from the receipt and/or use of Culti-Loops. Oxoid is not liable for damages arising from the misidentification or misrepresentation of strains.

Reference
1 Prier J., Bartola E. and Friedman H. (1973) *Quality Control in Microbiology. University Park Press, Baltimore.*

The current range of Culti-Loops may be found in the separate Oxoid Product List.

OXOID QUANTI-CULT$^{PLUS(TM)}$

This range features convenient ready-to-use preserved micro-organisms for use in quality control procedures. Each delivers a specific range of colony forming units (CFUs) and may be used for:

Growth promotion

Bacteriostasis and fungistasis

Testing of antimicrobial preservatives and disinfectants

Microbial limit tests

Media quality control etc.

Use
This product contains a specified number of preserved micro-organisms for use in quality control procedures[1,2,3]. Colony forming units (CFUs) are specified under defined procedures and growing conditions and cannot be guaranteed under other conditions. Results may vary with more inhibitory or selective media or with the same medium if of inferior quality.

Description
Each set consists of two vials packaged in a plastic bag. One vial, sealed within a silvery mylar envelope, contains a film of micro-organisms attached to the inside of the cap of the vial. The microbial film can be seen through the red cap as a black area inside the black 'O' ring of the cap. Rehydrating fluid is provided in the second vial.

Precautions
QUANTI-CULT$^{PLUS(TM)}$ contains live micro-organisms and should be used only by individuals with microbiological training. Properly disinfect any spills and sterilise used containers by autoclaving before final disposal.

Storage
Both rehydrating fluid and micro-organisms should be stored at refrigerator temperature (2–8°C) until time of analysis.

Expiration date
The reagents are stable through the expiration date on the label when stored as directed.

Evidence of deterioration
Each RED CAP should contain an intact dried film. Do not use if the film has come out of the cap or shows evidence of hydration.

To open
The foil envelope can be cut open with scissors.

To rehydrate
CAUTION
THE REHYDRATED SUSPENSION MUST BE USED WITHIN 30 MINUTES AFTER THE 10 MINUTE REHYDRATION PERIOD. DO NOT PLAN TO USE A REHYDRATED SAMPLE THROUGHOUT THE WORK DAY OR THE FOLLOWING DAY. PROLONGED HOLDING WILL ADVERSELY EFFECT THE NUMBERS OF VIABLE ORGANISMS.

1 Remove vials from the refrigerator. Place the rehydrating fluid vial (blue cap) in a 35 to 37°C incubator to warm.
2 Allow the vial (red cap) containing the microbial film (silver envelope) to warm to room temperature.
3 Remove the vial (red cap) containing the microbial film from the envelope.
4 Remove and discard the blue cap from the rehydrating fluid.
5 Remove the red cap containing the micro-organisms and transfer to the rehydrating fluid vial. Tighten cap.
6 Invert this vial, tap to be sure liquid is in contact with the inside of the cap, and place in 35 to 37°C incubator in an INVERTED position for 10 minutes* to dissolve the preserved micro-organisms. Autoclavable polyester racks may be used to hold the vials in an inverted position. Please note that the foam is an efficient insulating material and will retard warming or cooling of liquids.
7 Grasp bottom of vial, hold firmly in an inverted position, tap cap gently to mix suspension and aid dissolution. Excessive vigorous shaking will produce foam.

*CAUTION
Look at the cap to make certain that all of the micro-organisms are in solution. Undissolved intact black particles can be seen through the plastic cap and/or vial. If this happens, reinvert the vial and place back in the incubator and observe closely every 1 to 2 minutes for complete dissolution. Undissolved microbial film will cause reduced counts but prolonged heating may also result in incorrect counts.

Inoculation and sample analysis
QUANTI-CULT$^{PLUS(TM)}$ is designed to deliver ten 100µl inocula each containing between 10 and 100 CFUs from a single source.

QUANTI-CULT$^{PLUS(TM)}$ is suitable for evaluation of culture media used for the growth promotion and/or bacteriostasis and fungistasis procedures[1], other media[3], or quality control of any quantitative microbiological procedure[2].

CAUTION
THE REHYDRATED SUSPENSION MUST BE USED WITHIN 30 MINUTES AFTER THE 10 MINUTE REHYDRATION PERIOD. DO NOT PLAN TO USE A REHYDRATED SAMPLE THROUGHOUT THE WORK DAY OR THE FOLLOWING DAY. PROLONGED HOLDING WILL ADVERSELY EFFECT THE NUMBERS OF VIABLE ORGANISMS.

Hold the rehydrated suspension at room temperature throughout the 30 minute use period. Re-mix sample between withdrawals. When inoculating or transferring to a liquid matrix, it is advisable to have the receiving liquid pre-warmed to 35–37°C. Dispensing into a cold liquid may interfere with even distribution of the micro-organisms.

References

1 United Sates Pharmacopoeia XXII 1990, Mack Publishing Co.

2 Clesceri L.S., Greenberg A.E. & Trussel R.R. eds. 1989. *Standard Methods for the Examination of Water and Wastewater. 17th ed. A.P.H.A., A.W.W.A. & W.P.C.F.*

3 Mehlman I.J. 1984. *Appendix I. Culture media. In Bacteriological Analytical Manual. A.O.A.C. Arlington, VA.*

QUANTI-CULT$^{PLUS(TM)}$

Each tube contains 10 packs (10 tests per pack). Each kit is designed to deliver between 10–100 cfu/ 0.1ml.

Total diluent volumes per vial is 1.2ml.

Note
The organisms used in QUANTI-CULT$^{PLUS(TM)}$ are derived from original ATCC stock cultures according to the number shown.

QUANTI-CULT$^{PLUS(TM)}$ are manufactured for Oxoid Ltd by Chrisope Technologies Inc., an FDA approved company.

FDA Registration No: 1625984. QUANTI-CULT$^{PLUS(TM)}$ is a registered trademark of Chrisope Technologies Inc, a division of remel.

The current range of Quanti-Cult may be found in the separate Oxoid Product List.

3
PEPTONES, HYDROLYSATES, AGARS & CONSTITUENTS

November 1998

LABORATORY PREPARATIONS

Oxoid Laboratory Preparations are Culture Media reagents which are either:

(i) manufactured within Oxoid to specified quality performance standards

(ii) manufactured outside for Oxoid to the same high standards

(iii) selected from screened buying samples by extensive laboratory testing.

The L-P range includes peptones, protein hydrolysates, biological extracts, agars and the critical culture media chemicals such as selective agents, dyes etc.

Products are provided for users who wish to create their own media or who wish to supplement existing formulae. It should be stressed, however, that the use of these products will not necessarily reproduce the performance of listed Oxoid Culture Media, even used in identical formulae. This is because it is impossible to produce peptones or hydrolysates which can be universally applied to any formulae.

3.1 Peptones, hydrolysates and biological extracts.

3.2 Agars.

3.3 Bile, bile salts and derivatives.

3.4 Chemicals for culture media.

For bulk users Oxoid can manufacture laboratory preparations to meet special requirements.

PEPTONES, HYDROLYSATES AND BIOLOGICAL EXTRACTS

1 Introduction

2 Basic Information
- *i Biochemistry of Proteins*
- *ii Hydrolysis of Proteins*
- *iii Manufacture of Peptones*
- *iv Quality Assurance*

3 Meat Peptones
- *i Liver Digest Neutralised Code L27*
- *ii Peptone Bacteriological Code L37*
- *Peptone Bacteriological Neutralised Code L34*
- *iii Mycological Peptone Code L40*
- *iv Tryptose Code L47*
- *v Peptone P Code L49*
- *vi Special Peptone Code L72*
- *vii Proteose Peptone Code L85*

4 Vegetable Peptone
- *i Soya Peptone Code L44*

5 Casein Peptones and other milk derived Peptones
- *i Peptonised Milk Code L32*
- *ii Casein Hydrolysate (Acid) Code L41*
- *iii Tryptone Code L42*
- *iv Tryptone T Code L43*
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6 Extracts
- *i Yeast Extract Code L21*
- *ii Lab-Lemco Code L29*
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7 References

8 Packaging & Services

9 Table of Analysis
- *i General Analysis*
- *ii Amino Acid Analysis*

10 Uses of Oxoid Peptones, Hydrolysates and Biological Extracts

1 INTRODUCTION

The first time the term 'peptone' appeared was in papers published in 1880 and 1882 by Nageli. He has been credited as the first bacteriologist to discover that chemo-organotrophic organisms grow best in culture media containing a partially digested protein.

The problems associated with the production of protein hydrolysates were quickly recognised and their manufacture became the concern of commercial suppliers. In fact protein hydrolysate was the first complex culture medium ingredient to be supplied commercially. This was the fore-runner of the large range of commercial culture media now available.

Oxoid (then the Medical Division of Oxo) started its investigation into the manufacture of peptone in 1924.

The variety of peptones and extracts available reflects the differing demands of micro-organisms for amino acids, peptides and other nutrients. Substrates used by Oxoid for hydrolysis include: meat, casein, lactalbumin, milk, gelatin, soya and yeast cells.

2 BASIC INFORMATION

Biochemistry of Proteins
Proteins are macro-molecules and are fundamental to the structure and function of all living organisms. Chemically, proteins are made up of one or more chains of alpha-amino-carboxylic acids (amino acids), consecutively linked covalently between the alpha-amino group of one moiety and the alpha carboxylic group of the next with the elimination of water. This linkage is termed the 'peptide bond'. Chains of three or more amino acids are termed 'polypeptides', whilst larger structures, with an arbitrarily determined lower molecular weight limit of 5,000 are the proteins. An example of a Peptide and Amino Acid is shown in Figure A.

Only 20 amino acids commonly occur in proteins. They can occupy any position in the protein chain which can be at least 80 units long with molecular weights of several millions. The chains are folded in a variety of complex forms and the structures may incorporate other macro-molecules such as carbohydrates and lipids.

Hydrolysis of Proteins
The hydrolysis of proteins, which breaks them down to their constituent amino acids and peptides can be achieved by the use of strong acids, strong bases or proteolytic enzymes such as pepsin, papain and pancreatin (which contains trypsin)[1].

Hydrolysis with strong mineral acids, often at high temperatures and pressures is much used in the food industry to produce food flavourings. The most commonly used product in microbiology is based on the hydrolysis of casein. In this process all peptide bonds are attacked and in theory, complete breakdown into component parts could be obtained. However, because the reaction conditions are so severe, some of the amino acids produced are themselves destroyed by the process, notably tryptophan which is totally lost. Cystine, serine and threonine are partially broken down but asparagine and glutamine are converted to their acidic forms. Any vitamins present are largely destroyed. A series of reactions may also take place between carbohydrates and amino acids (such as the Maillard reactions) which give rise to very dark products often toxic to the growth of micro-organisms[2]. For microbiology the amount of hydrolysis is controlled to produce a suitable nitrogen source for bacteria.

Proteolytic enzymes act on proteins under less severe conditions. They will function at much lower temperatures and at normal pressures and are usually specific to the peptide bond they will attack. This means that the protein is not completely hydrolysed to its constituent amino acids but into polypeptides of varying lengths, depending on the frequency of the specific amino acid linkage. Also, since proteins have a very consistent primary structure, the mixture of peptides produced after proteolytic digestion by a specific enzyme is also consistent.

Enzymes commonly used are papain, pepsin and pancreatin, Figure A.

Pepsin will cut the chain anywhere there is a phenylalanine or leucine bond[3].

Papain cuts adjacent to arginine, lysine, phenylalanine and glycine[4].

Pancreatin has its action at arginine, lysine, tyrosine, tryptophan, phenylalanine and leucine bonds[4].

Raw materials may vary considerably in composition and the extent to which the protein components have been denatured during any processing procedures, therefore the conditions of manufacture must be carefully controlled to minimise the variations inherent in biological materials and so maintain quality.

More defined protein sources, such as casein and gelatin will give more consistent mixtures of peptides when treated with enzymes or acid.

In practical terms, total breakdown of a protein to its individual component amino acids is difficult even with a mixture of enzymes; the result, even with well defined proteins such as casein, is a peptone containing a chemically undefined mix of peptides and amino acids.

Manufacture of Peptones
The manufacturing process is illustrated diagrammatically in Figure B and the syrup formed can be stored for long periods at room temperature because the high dissolved solid content inhibits bacterial contamination. This syrup can be used in fermentation processes without drying to a powder.

Quality Assurance
It is essential that the quality of these products is maintained at the highest level and lot to lot variation reduced to a minimum by closely following codes of Good Manufacturing Practice (GMP)[5]. In order to achieve this several types of analysis are carried out and strict quality control specifications must be met for a lot to be accepted. A list of average analyses of hydrolysed products is shown on page 3–12.

To ensure that the product conforms to predetermined specifications tests are carried out and the following criteria are routinely monitored: clarity and colour, moisture content, pH value, ash residue, chloride, nitrogen content and microbiology.

Clarity and pH Value
These tests are performed on an autoclaved 2% solution of the final product and are controlled by comparison with reference materials.

Moisture Content
The level of moisture should be below 5% to ensure no chemical changes occur if the product is stored at high ambient temperatures.

Ash Residue
The ash residue consists mainly of inorganic material and is estimated after ignition.

Chloride
Chloride content is determined using the Volhard titration method on the ash residue.

Metal Analysis
The presence of cations, such as calcium and magnesium, is often of value to organism growth, since they contribute significantly through their roles as co-factors in key metabolic pathways. Consequently, these are routinely measured by atomic absorption spectroscopy, to ensure control and consistency in the final products.

Total Nitrogen
An important measure of any hydrolysate or extract is its nitrogen content. Investigations are carried out to ascertain the total nitrogen (TN) which is measured by the Kjeldhal digestion and titration method[6].

To calculate the % protein, peptide or amino acid present multiply %TN by 6.25. This is approximate because of the other sources of nitrogen in peptones such as nucleotides.

Figure A Diagram of enzymatic action

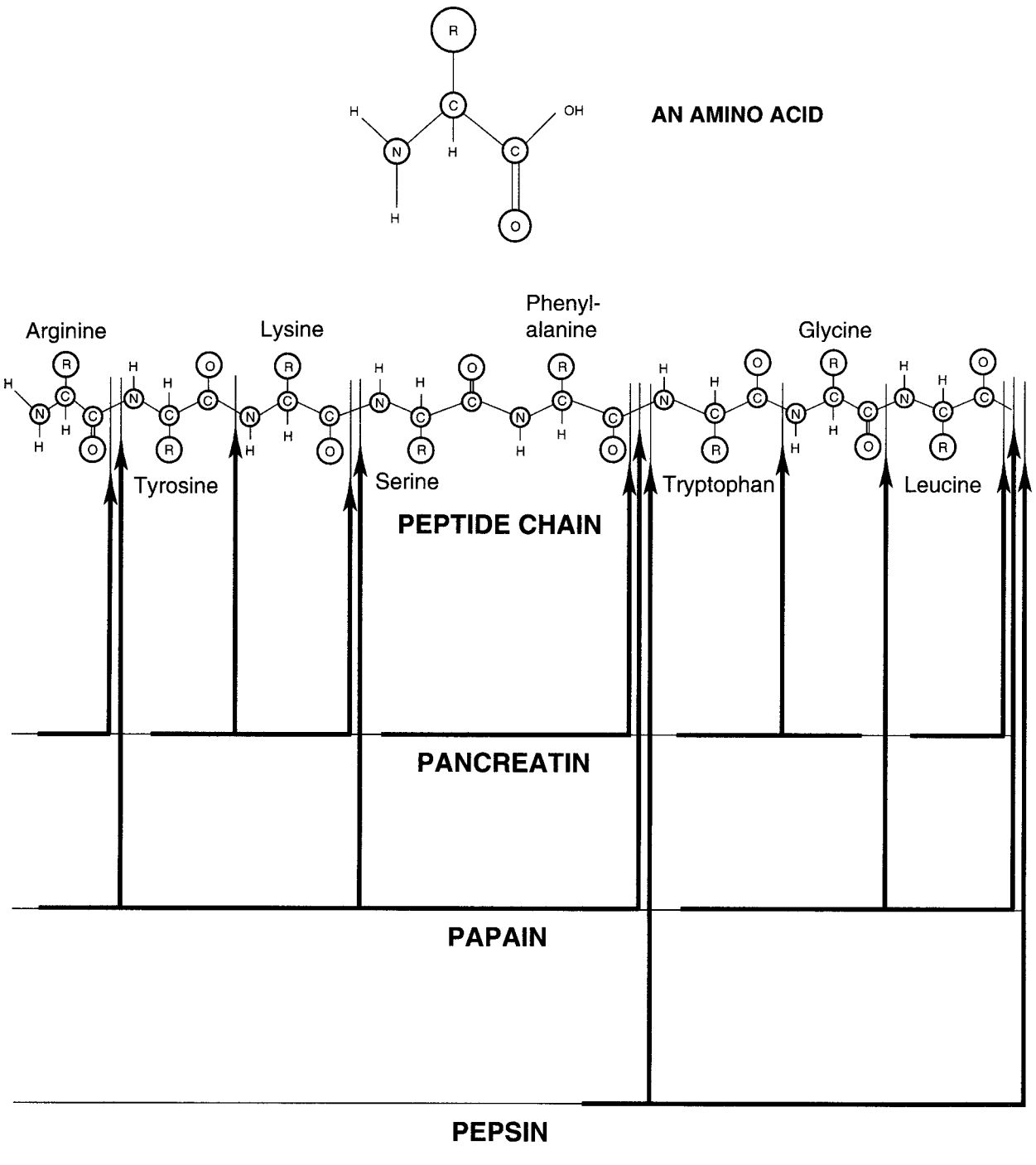
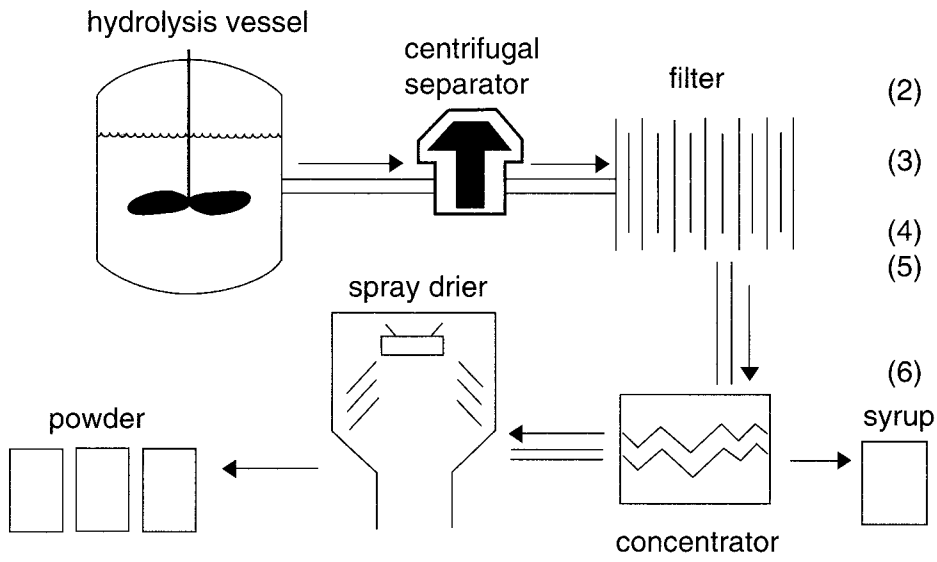


Figure B Diagram of Manufacturing Process



(1) Protein as fresh meat or powder added with demineralised water to form a slurry.
(2) Digested with acid or enzyme.
(3) Centrifuge to remove fat or oil.
(4) Filter.
(5) Concentrate to approximately 67% total solids (syrup).
(6) Spray dry to powder.

Amino Nitrogen
A second investigation of nitrogen content measures the amino nitrogen (AN) also by a titration method which reacts only with amino groups of peptides and amino acids[7].

The amino nitrogen titration shows the extent of hydrolysis by measuring the increase in free amino groups from the protein. The greater the percentage of AN the greater the degree of digestion.

Degree of Hydrolysis
The degree of hydrolysis (D_H)[8] is measured by the number of peptide bonds cut, divided by the total number of peptide bonds, multiplied by a hundred and is calculated by the formula:

$$\%D_H = \frac{\text{AN Peptone} - \text{AN Protein} \times 100}{\text{TN Protein}}$$

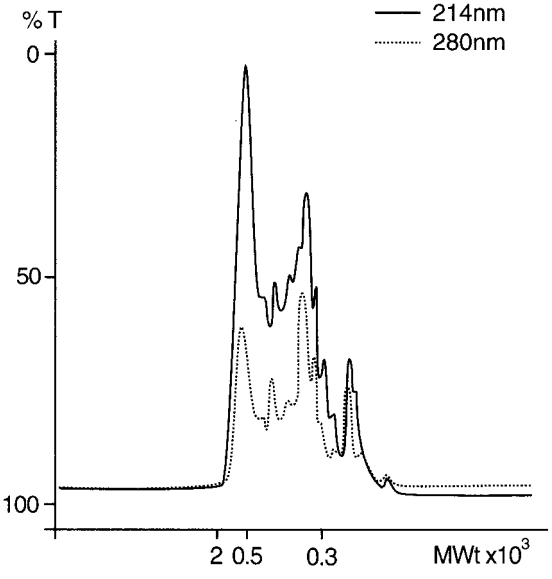
An approximate chain length of the hydrolysate can be derived by dividing 100 by the D_H, e.g. if the D_H of Casein Hydrolysate (Acid) L41 is 22% then $^{100}/22$ = 4.55 which is the average peptide chain length (ACL).

Total Amino Acids
Another indication of the potential nitrogen availability is the total amino acid profile, which is determined by High Pressure Liquid Chromatography (HPLC). This data is the result of the complete breakdown of the polypeptides to their constituents and their subsequent analysis. If a micro-organism was able to repeat this reaction biochemically, then it would have the spectrum of amino acids recorded in the Table of Analysis available for assimilation and utilisation. In reality, it is the spectrum of peptides which are of more value to the organism than the amino acids and these can be analysed by different techniques.

Molecular Weight Profile
Moelcular size information can be obtained from analytical data and gives a useful indication of the amount of hydrolysis the substrate underwent, or degree of digestion. Using HPLC, the method of Size Exclusion Chromatography reveals the distribution of polypeptides and amino acids present in the peptone. Peptides of high molecular weight are eluted first and the smaller amino acids elute later. In the examples of the profiles below, the X axis represents elution time, or volume of mobile phase eluted and the Y axis represents the detection wavelength. This gives an indication of the amount and type of component present. At 280nm only those peptides containing aromatic amino acid residues are observed, whereas at 214nm a wider range of peptides are detected. (Figure C).

Casein Hydrolysate (Acid) has a high D_H as acid breaks peptide bonds indiscriminately. Tryptone is casein hydrolysed with pancreatin and as this enzyme

Figure C Molecular profile of L37 Bacteriological Peptide at different detection wavelengths



has its action at specific bonds, less hydrolysis is the result. Proteose peptone is specially digested to contain higher molecular weight peptides and so has the lowest D_H of all. From work by Adler-Nissen (ref 8) Figure D shows that during the course of a digest the D_H achieved depends on a number of factors such as enzyme concentration or hydrolysing agent used. Other variables that affect D_H include type of substrate, temperature and pH.

Size exclusion is perhaps one of the most useful analyses of protein hydrolysates and assists in the development of new products while helping to maintain quality and reproducibility of existing processes. The peptone profiles above show the affect of hydrolysis time on the molecular profile (Figure E). Thus a range of peptones can be made with a wide variety of chemical and bacteriological properties to different specifications.

Figure D Effect of Enzyme Concentration and Type of Hydrolysis on D_H

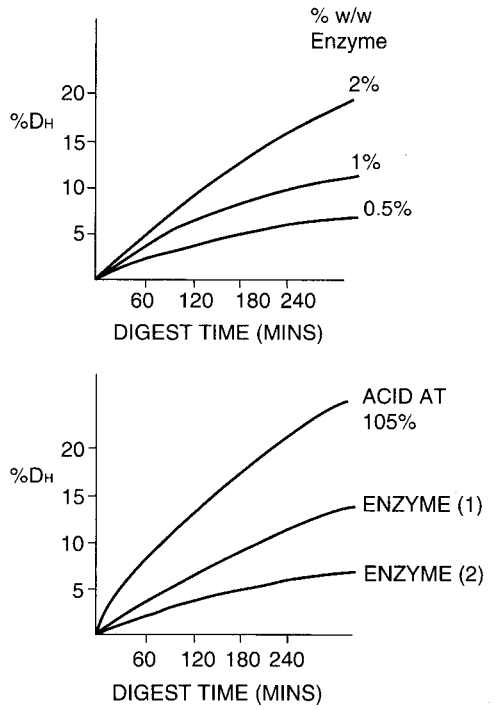
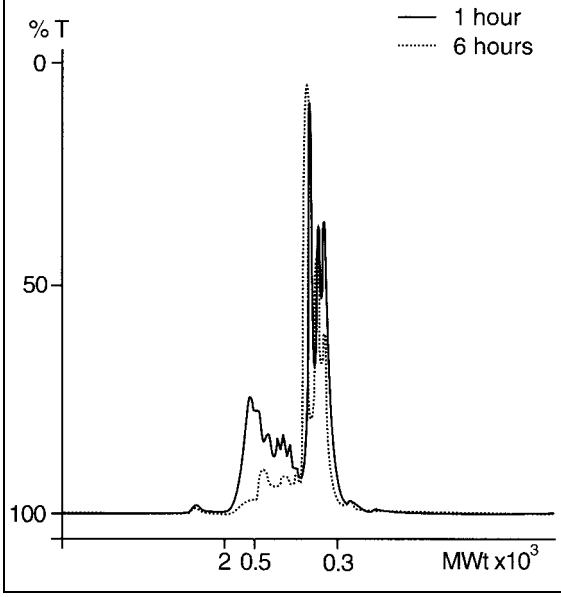
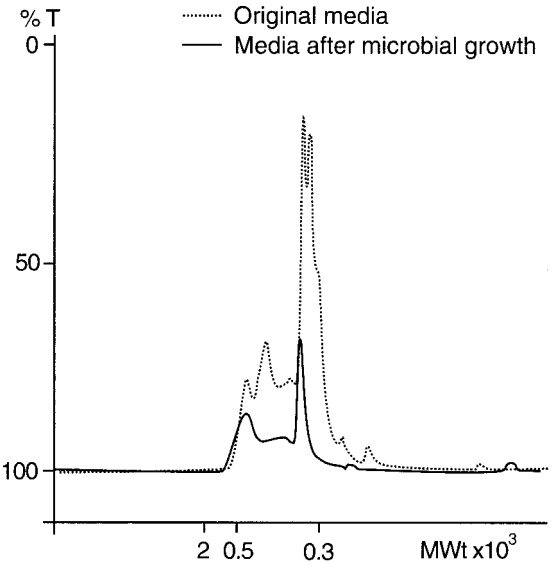


Figure E Change in Molecular Profile of typical Peptone with hydrolysis time



How the Test can Help the End User
Molecular profiles can give valuable information about the user's application and particularly shows how to improve yields or growth of organisms. Profiles can be run on peptones or complete fermentation media before and after microbial growth. By making a comparison of the profiles before and after use, an indication of the efficiency of the peptone for growing a particular organism is obtained. These peptones can then be modified to maximise organism growth or product yield. An example of the peptone utilisation is shown in Figure F.

Figure F Profiles of Fermentation Broths



3 MEAT PEPTONES

Oxoid manufactures a comprehensive range of Meat Peptones derived from different animal tissues to suit a range of nutritional requirements, using a number of proteolytic enzymes and manufacturing processes. Since the origin of these animal materials is important, Oxoid only source from countries whose disease status is acceptable and only use tissues from selected portions of the animal. The tissues are hydrolysed to produce straw coloured peptones which are highly nutritious and clearly soluble in water. The product reaches the consumer as an easily handled, spray dried powder, although for some applications the product can be used in syrup form.

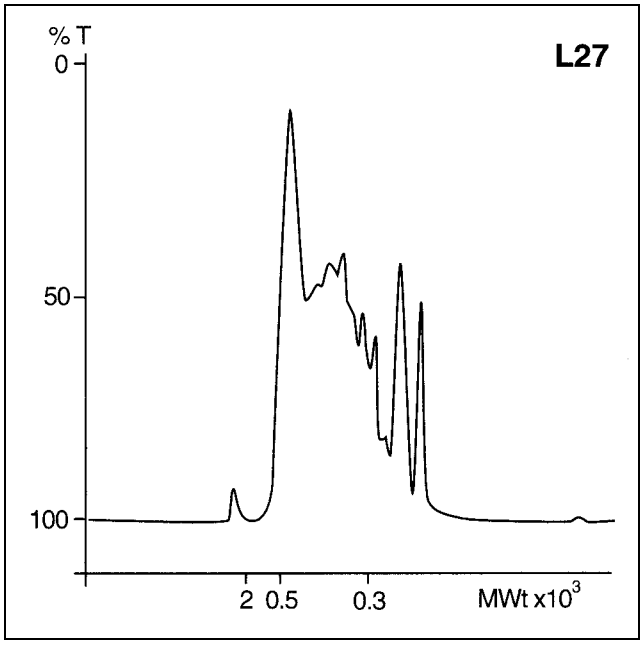
LIVER DIGEST NEUTRALISED

Code: L27

A biologically standardised papaic digest of liver for use as a source of nutrients in microbiological culture media.

The digest is water soluble and compatible with other culture media ingredients and may be sterilised by filtration or autoclaving; thus it is suitable for use as an integral part of many culture media or as a valuable supplement. Being derived from liver this product contains relatively high levels of iron. The profile shows the characteristic even spread of peptides obtained from papaic digests.

Typical Analysis (% w/w)	
Total Nitrogen	11.0
Amino Nitrogen	3.6
Sodium chloride	1.6
pH (2% solution)	7.1



PEPTONE BACTERIOLOGICAL

Code: L37

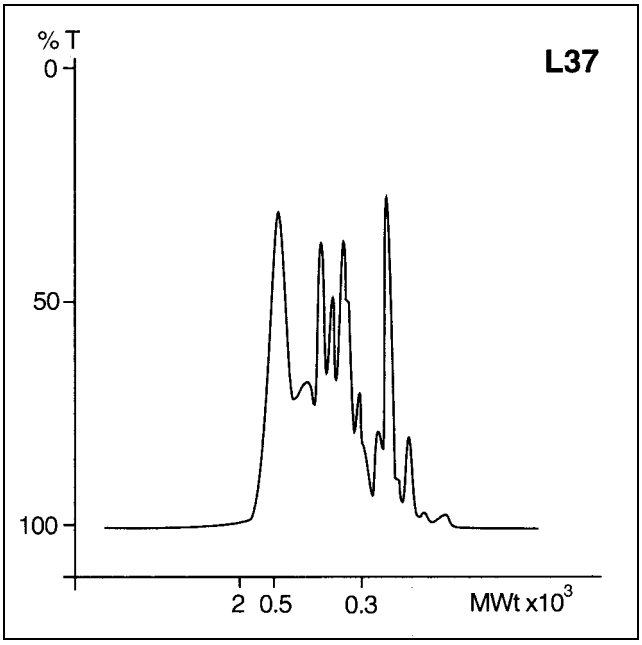
PEPTONE BACTERIOLOGICAL NEUTRALISED

Code: L34

Oxoid Peptone Bacteriological and its neutralised form are very nutritious all-purpose peptones prepared by the enzymatic digestion of selected animal protein sources. They are specially prepared to provide a solid foundation in culture media formulations and are compatible with other refined culture media ingredients. The combination of pancreatin and papain enzyme systems ensures that these bacteriological peptones contain a wide spectrum of polypeptides, reflected in their broad molecular profiles.

The neutralised form evolved from the original to meet those occasions when a slightly higher pH is required. Both when reconstituted give a solution free of haze, cloudiness or precipitation.

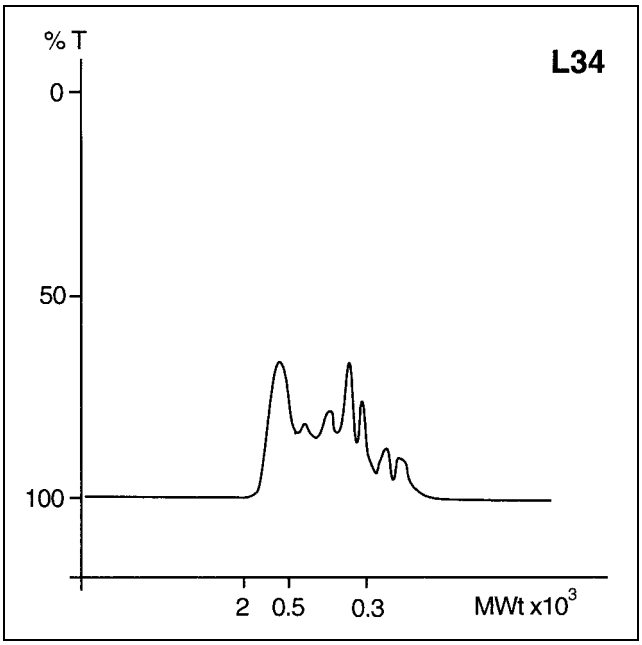
L37 Typical analysis (% w/w)	
Total Nitrogen	15.2
Amino Nitrogen	2.9
Sodium chloride	1.0
pH (2% solution)	6.3



L34 Typical analysis (% w/w)	
Total Nitrogen	13.9
Amino Nitrogen	2.4
Sodium chloride	3.2
pH (2% solution)	7.2

Either may be used wherever a high quality bacteriological peptone is called for. Both products are found in a wide range of culture media in routine diagnostic and research bacteriology.

The above products are used in industry to produce antibiotics, interferon, pasteurella vaccine and as a stabiliser for other vaccines.



MYCOLOGICAL PEPTONE

Code: L40

Mycological Peptone was developed specifically for incorporation in solid media used for the isolation and diagnosis of pathogenic and non-pathogenic fungi. It rapidly gives a luxuriant growth with typical morphology and pigmentation. Since it does not encourage bacterial growth because of its acidity media containing this peptone are useful for the isolation of pathogenic fungi from material heavily infected with bacteria.

It is a blend of peptones with a pH of 5.4.

Typical analysis (% w/w)	
Total Nitrogen	9.5
Amino Nitrogen	2.9
Sodium chloride	1.3
pH (2% solution)	5.4

TRYPTOSE

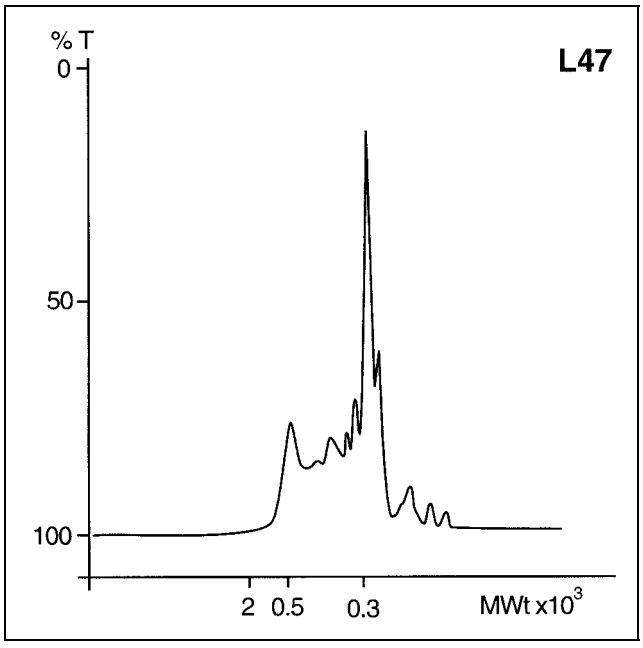
Code: L47

Tryptose is a mixed enzymatic hydrolysate with unique nutritional properties.

The digest conditions are such that it contains many different peptides including those of higher molecular weight (proteoses). It is used to grow the most fastidious of organisms especially when a rapid or profuse growth is required e.g. in blood culture media. Tryptose is also recommended to demonstrate haemolytic reactions on a blood agar base.

It is used in the production of foot and mouth disease vaccine.

Typical analysis (% w/w)	
Total Nitrogen	13.7
Amino Nitrogen	3.2
Sodium chloride	1.0
pH (2% solution)	7.2



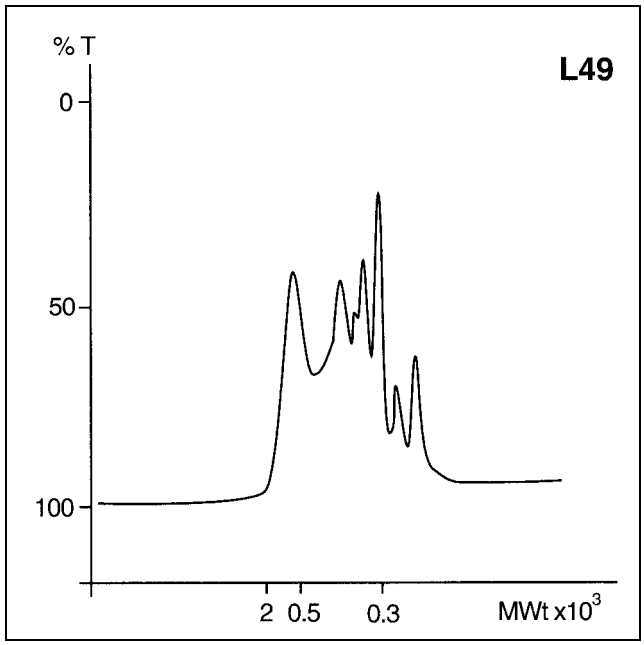
PEPTONE P

Code: L49

A peptic digest of meat proteins used in bacteriological culture media which complies with the USP Specification[9] for peptic digest of animal tissue. The molecular profile shows the characteristics of peptic hydrolysates, having a shift to higher molecular peptides and the salt content reflects the low pH required for the optimum activity of the enzyme during processing.

It has been used as a replacement for bovine serum in a medium on which Baby Hamster Kidney (BHK) cells were grown. Also incorporated in media to produce interferon.

Typical analysis (% w/w)	
Total Nitrogen	12.8
Amino Nitrogen	2.8
Sodium chloride	9.3
pH (2% solution)	7.0



SPECIAL PEPTONE

Code: L72

A specially designed mixture of peptones, including meat, plant and yeast digests designed to encourage the growth of the most demanding organisms. It contains a wide spectrum of peptide sizes together with those minerals, vitamins, nucleotides and other carbon compounds present in the individual peptones.

Special Peptone is an ingredient of media where a wide range of fastidious organisms are to be cultured such as, Columbia Agar or Schaedler media and GC Agar Base.

Typical analysis (% w/w)

Total Nitrogen	12.2
Amino Nitrogen	3.5
Sodium chloride	3.5
pH (2% solution)	7.2

PROTEOSE PEPTONE

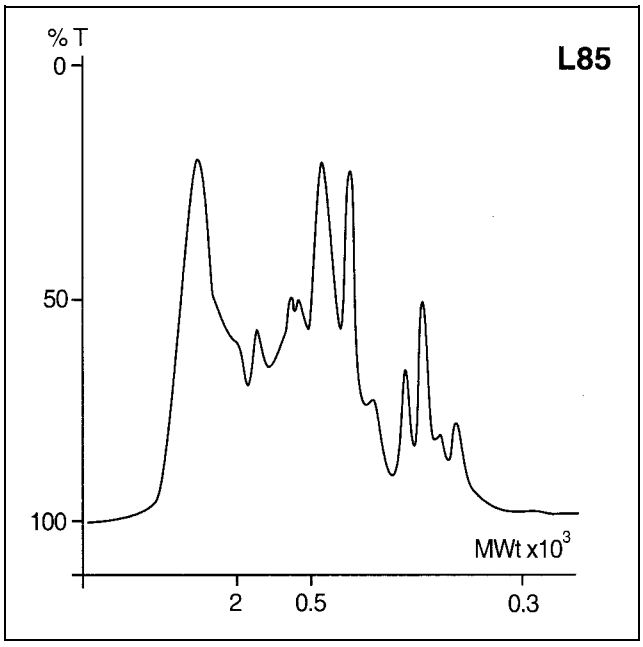
Code: L85

A specialised peptone prepared from a mixture of peptones. This product contains proteoses as defined in the United States Pharmacopoeia[9]. This has been achieved by carefully controlling manufacturing conditions to achieve a product rich in the higher molecular weight peptides, (e.g. 4000 plus).

Proteose Peptone is especially suitable in media for *Corynebacterium diphtheriae* toxin, including that for the Elek reaction for the recognition of toxigenic strains, as well as in the media for the production of toxins from staphylococci, clostridia and salmonellae. Media incorporating this peptone are suitable for the cultivation of different bacteria with a wide range of nutritional requirements, e.g. *Neisseria*, *Staphylococcus*, *Haemophilus*, *Salmonella*, *Pasteurella*, *Corynebacterium* and *Histoplasma* species.

It is the peptone used to manufacture diptheria toxoid, pertussis vaccine and measles vaccine.

Typical analysis (% w/w)	
Total Nitrogen	13.0
Amino Nitrogen	2.2
Sodium chloride	8.0
pH (2% solution)	7.0



4 VEGETABLE PEPTONE

SOYA PEPTONE

Code: L44

Soya Peptone is obtained by the papain hydrolysis of soya flour and complies with the USP specification (ref 9). In additon to its nitrogen constituents, this peptone has a high carbohydrate content and is suitable for many purposes. The presence of the sugars stachyose, raffinose, sucrose and various reducing sugars may be of importance in certain applications. It is widely used in culture media and is often used for the cultivation of many fastidious organisms and where rapid, luxuriant growth is required.

Typical analysis (% w/w)	
Total Nitrogen	9.1
Amino Nitrogen	2.3
Sodium chloride	0.4
pH (2% solution)	7.2

5 CASEIN AND OTHER MILK DERIVED PEPTONES

Oxoid manufactures a range of casein peptones and milk derived peptones sourcing the raw materials from countries whose disease status is acceptable.

By careful control of the hydrolysis conditions, products with widely differing physical, chemical and microbiological characteristics have been achieved.

Hydrolysis with acid is non specific, attacking all peptide bonds and degrading proteins and polypeptides to low chain length peptides and amino acids. In the analytical data this is shown by a high amino nitrogen (compared to enzymatic digests) and the presence of high levels of sodium chloride. The latter is caused by neutralisation of hydrochloric acid with sodium hydroxide.

Acid hydrolysis destroys tryptophan and partially destroys cystine, serine and threonine. Asparagine and glutamine are converted to their acidic forms and vitamins are destroyed.

Lactalbumin Hydrolysate, Peptonised Milk, Tryptone and Tryptone T are made by less severe forms of hydrolysis using pancreatic enzymes on various milk fractions.

PEPTONISED MILK

Code: L32

This is a pancreatic digest of high grade skimmed milk powder. It constitutes a source of nitrogen more readily available than milk or milk powder and has a high level of carbohydrate. As with milk powder, the calcium level is relatively high.

The product may be used on its own or in conjunction with other ingredients in media for isolation of lactobacilli and bacteriological examination of dairy products.

It has a high tryptophan content and is therefore used in media for testing the indole reaction.

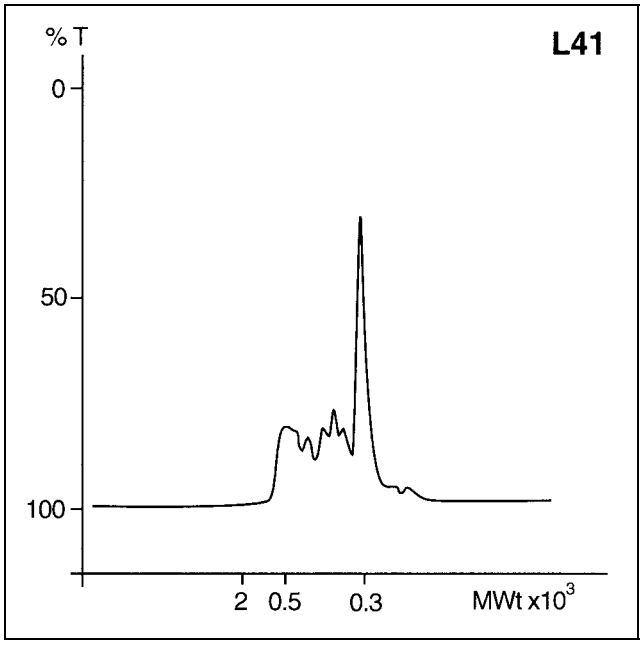
Typical analysis (% w/w)	
Total Nitrogen	5.3
Amino Nitrogen	1.8
Sodium chloride	1.6
Tryptophan	0.53
pH (2% solution)	6.5

CASEIN HYDROLYSATE (ACID)

Code: L41

An hydrolysate prepared by the reaction of casein with hydrochloric acid at high temperature and pressure, followed by neutralisation with sodium hydroxide. The aggressive hydrolysis conditions require specialised processing to decolorise, to achieve a light coloured peptone. The high availability of amino acids in their native form is advantageous in many culture media formulations and the molecular profile shows a definite shift to the lower molecular spectrum. It has particular characteristics which make it compatible for use in sensitivity media and those applications where salt tolerant organisms are used.

Typical analysis (% w/w)	
Total Nitrogen	8.2
Amino Nitrogen	5.3
Sodium chloride	30.2
pH (2% solution)	7.0



TRYPTONE

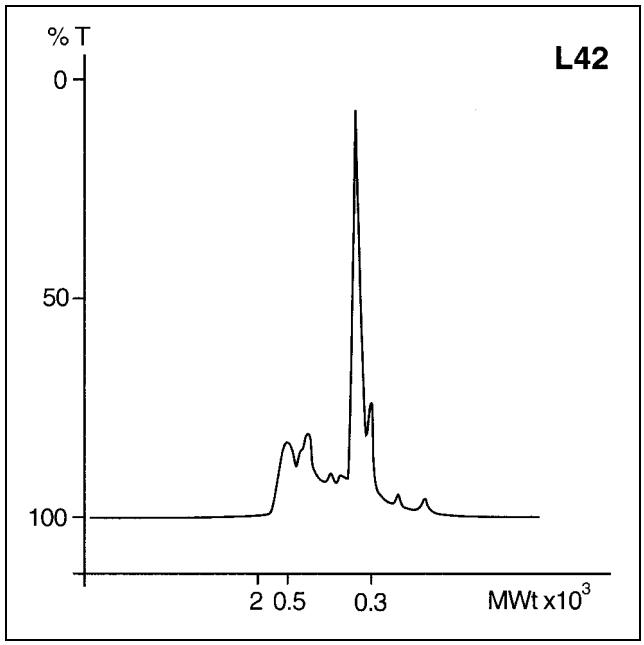
Code: L42

Tryptone is a pancreatic digest of casein. It can be used in any formulation where a pancreatic or tryptic digest of casein is specified and complies with the specification for pancreatic digest of casein in the U.S. Pharmacopoeia[10]. Casein is the main protein of milk and is a rich source of amino acid nitrogen. The profile shows a broad spread of peaks throughout the molecular weight range characteristic of a pancreatic digest. This hydrolysate is often mentioned in published works, either as a constituent of culture media for metabolic or growth studies, or for other purposes where high performance and uniformity of composition are of paramount importance. It has a high tryptophan content and is therefore used in media for testing the indole reaction.

Tryptone can detect 'flat-sour' or 'sulphide' spoilage organisms in the canning industry and is also used in sterility testing media.

It is a constituent of media used in fermentation processes to produce antibiotics, extra-cellular protein, interferon and diphtheria toxoid.

Typical analysis (% w/w)	
Total Nitrogen	13.3
Amino Nitrogen	3.7
Sodium chloride	0.4
pH (2% solution)	7.3



TRYPTONE T

Code: L43

This product was developed from Oxoid Tryptone L42 by controlled enzymatic hydrolysis and modified by the method of Meuller and Miller[11]. This produces a lower level of calcium, magnesium and iron than in Tryptone which makes it ideal for the production of toxin by *Clostridium tetani*.

Typical analysis (% w/w)	
Total Nitrogen	11.7
Amino Nitrogen	3.5
Sodium chloride	3.5
Calcium	280ppm
Magnesium	24ppm
Iron	3ppm
pH (2% solution)	7.0

LACTALBUMIN HYDROLYSATE

Code: L48

After removal of casein from milk, lactalbumin is a protein extracted from the resulting whey. L48 is a pancreatic digest of this protein and contains high levels of essential amino acids.

It is most commonly used in media for tissue culture and therefore production of vaccines of viral origin including foot and mouth disease, polio, dengue, coxsackie B3 and many other viruses.

Other uses include growth of lactobacilli, spore growth of clostridia and in fermentation procedures for hormone production.

Typical analysis (% w/w)	
Total Nitrogen	12.5
Amino Nitrogen	5.4
Sodium chloride	0.2
pH (2% solution)	6.5

6 EXTRACTS

YEAST EXTRACT

Code: L21

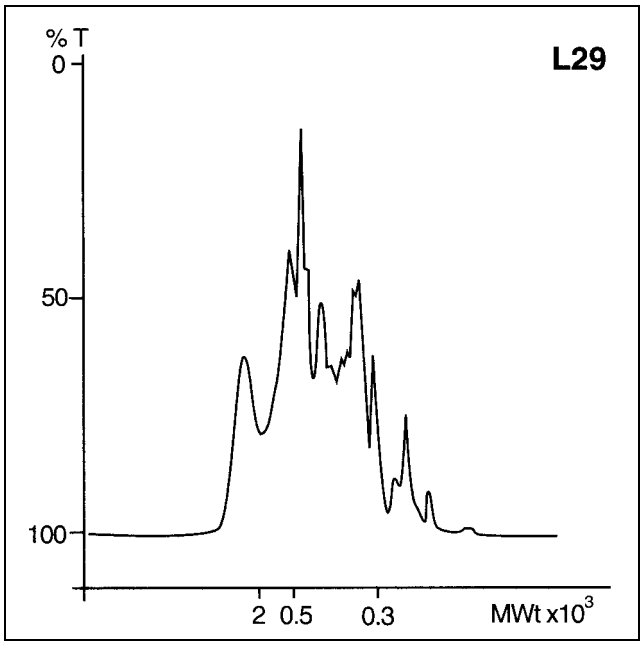
This is a dried yeast autolysate which is a good source of amino-nitrogen and vitamins, particularly the water soluble B-complex vitamins. Its addition to many media or fermentation broths increases the yield of organisms and is recommended where rapid and luxuriant growth is required.

Typical analysis (% w/w)	
Total Nitrogen	10.9
Amino Nitrogen	5.3
Sodium chloride	0.3
pH (2% solution)	7.0

LAB-LEMCO

Code: L29

Lab-Lemco is a meat extract made from specially selected raw materials, adjusted to neutrality and dried to a fine powder. The product has considerable advantages over conventional meat extracts. Being a refined and clarified extract it can be used with other refined ingredients to make culture media which require no filtration. Being only slightly hygroscopic this product is very easy to handle. Its use eliminates the troublesome procedures associated with handling conventional meat extracts which have a paste-like consistency.



It will enhance the growth of many bacteria and is therefore incorporated into a wide range of culture media as a solid foundation material.

It is used in fermentation processes.

Typical analysis (% w/w)	
Total Nitrogen	13.3
Amino Nitrogen	2.5
Sodium chloride	1.1
pH (2% solution)	7.2

MALT EXTRACT

Code: L39

This is prepared by extracting the soluble products from sprouted grain followed by low temperature evaporation to dryness which conserves the nitrogenous and carbohydrate constituents.

It is recommended for use in media for the growth of yeast and moulds.

Typical analysis (% w/w)	
Total Nitrogen	1.1
Amino Nitrogen	0.6
Sodium chloride	0.1
pH (2% solution)	5.6

References

1 Haurowitz F. (1963)*'The Chemistry & Function of Proteins' 2nd Edition Academic Press.*
2 Einarsson H., Snygg B. G., Ericsson G. (1983) *J. Agric. Food Chem. 31. 10.*
3 Dixon M., Webb E. C. (1979) *'Enzymes'. 3rd Edition Longman, GP Limited, page 892.*
4 Dixon M., Webb E. C. (1979) *'Enzymes', 3rd Edition Longman, GP Limited, page 886.*
5 'Guide to Good Pharmaceutical Manufacturing Practice' (1983) *Editor J. Sharp. Her Majesty's Stationery Office.*
6 Bradstreet (1965) 'The Kjeldahl Method for Organic Nitrogen' *Academic Press, New York.*
7 Taylor (1957) *Analyst 82. 488.*
8 Adler-Nissen J. (1978) *Ann. Nutr. Alim. 32. 205–216.*
9 United States Pharmacopoeia (1985) *21st Revision p. 1396.*
10 United States Pharmacopoeia (1985) *21st Revision pp. 1394–1396.*
11 Meuller J. H. and Miller P. A. (1958) *J. Bact. 67. 271–277.*

7 PACKAGING AND SERVICES

All the products described are in the standard Oxoid range and are available in the normal sales pack of 250 grams or 500 grams. For large users they are supplied in 2.5K, 5K and 25K containers.

Some products are available in syrup form in 50K drums. If supplied in this state, the solution has solid equivalent of between 55% and 67% depending on the product. Such a syrup would usually represent the end stage of manufacture immediately before drying.

Lot sizes vary from product to product, between 450K to 3000K per lot.

Oxoid offer a sampling and reserve system whereby customers can reserve a bulk quantity of a product from which a sample is sent for them to test and after satisfactory results the same lot will be supplied.

Products not in our normal range, can be manufactured on request provided a minimum of 100K is required. Customers interested in this service are asked to state their specifications and discuss the feasibility of manufacturing any such product with their Oxoid contact.

LIVER DESICCATED

Code: L26

Dehydrated whole liver, specially manufactured for the preparation of infusion media. Liver Desiccated is prepared by the dehydration of fresh ox livers under carefully controlled conditions designed to ensure maximum retention of nutritive properties, and is equivalent to five times its weight of fresh liver.

To prepare a liver infusion medium, add 50 grams of Liver Desiccated to 1 litre of distilled water and allow to infuse (with frequent agitation) for 1 hour at 50°C. Boil the mixture for a few minutes to coagulate protein, strain through 60-mesh stainless steel gauze, add 10 grams of Peptone L34 and 5 grams of sodium chloride. Adjust the reaction to pH 7.2, boil, strain through gauze as above, and sterilise by autoclaving at 121°C for 15 minutes.

8 USES OF OXOID PEPTONES, HYDROLYSATES AND BIOLOGICAL EXTRACTS

Meat Peptones	**Culture Media**	**Antibiotic Manufacture**	**Fermentation**	**Tissue Culture**	**Vaccine Manufacture**	**Special Notes**
Neutral Liver Code L27	•					
Bact. Pept. Code L37	•	•	•		•	
Bact. Pept. Neut. Code L34	•	•	•		•	*Gives a clear solution*
Mycological Pept. Code L40	•					*Has low pH*
Tryptose Code L47	•			•	•	*Foot and mouth vaccine*
Peptone P Code L49	•		•			*Interferon production*
						Demonstrates H_2S
Special Peptone Code L72	•					*Fastidious organisms*
Proteose Peptone Code L85	•				•	*Diphtheria toxoid*
Vegetable Peptone						
Soya peptone Code L44	•	•	•			*Antibiotic production*
Casein and other Milk Derived Products						
Casein Hydrolysate (Acid) Code L41	•	•	•		•	*Antibiotic and vaccines*
Peptonised Milk Code L32	•					*Dairy investigations*
Tryptone Code L42	•	•	•		•	*Diphtheria toxoid, Antibiotics Interferon production*
Tryptone T Code L43					•	*Tetanus toxoid production*
Lact. Hydrolysate Code L48				•	•	*Spore formation Hormone production*
Extracts						
Yeast Extract Code L21	•	•	•		•	*Increases yield*
Lab-Lemco Code L29	•	•	•		•	*Easy to use meat extract*
Malt Extract Code L39	•					*Yeast and mould media*

TABLE OF ANALYSIS

GENERAL ANALYSIS

TYPICAL ANALYSIS (W/W) PEPTONE		% MOISTURE	% ASH	% AMINO NITROGEN (AN)	% TOTAL NITROGEN (TN)	AN/TN	$\%D_H$	pH	% NaCl	%K	Parts per million (ppm) Ca	Mg	Fe	Cu	Pb	Mn	Sn	Zn	Co
MEAT PEPTONES																			
LIVER DIGEST NEUTRALISED	L27	<5	10.6	3.4	11.4	30	28	7.0	2.2	1.6	98	210	245	12	0.2	1.2	2.9	127	0.2
BACTERIOLOGICAL PEPTONE	L37	<5	4.2	2.9	15.2	19	9	6.3	1.0	3.6	635	265	22	1	0.4	3.4	1.0	9.2	0.1
BACTERIOLOGICAL PEPTONE NEUTRALISED	L34	<5	9.6	2.4	13.9	17	9	7.0	3.2	1.4	180	300	77	16	0.3	0.9	1.2	9.2	0.2
MYCOLOGICAL PEPTONE	L40	<5	10.8	2.9	9.5	30	ND	5.4	1.3	2.6	125	845	190	60	0.3	1.0	5.8	56	0.2
TRYPTOSE	L47	<5	7.4	3.2	13.7	23	16	7.0	1.0	1.0	885	165	35	1	0.5	3.1	6.0	33	0.3
PEPTONE P	L49	<5	16.0	2.8	12.8	24	21	7.0	9.3	1.7	160	220	26	2	0.5	0.5	8.3	27	0.1
SPECIAL PEPTONE	L72	<5	11.4	3.5	12.2	29	33	7.2	3.5	2.0	580	530	51	2	0.8	6.0	1.8	38	0.7
PROTEOSE PEPTONE	L85	<5	13.8	2.2	13.0	17	16	7.0	8.0	1.4	200	340	42	10	<0.1	0.5	2.2	16	0.2
GELATIN PEPTONE	L92	<5	4.6	2.8	15.9	18	12	7.2	1.5	0.04	443	94	12	2	0.1	2.2	0.7	3.9	<0.1
VEGETABLE PEPTONE																			
SOYA PEPTONE	L44	5.8	13.5	2.3	9.1	25	29	7.2	0.4	3.3	225	1530	90	2	0.3	1.0	1.1	12	0.2
CASEIN AND OTHER MILK DERIVED PEPTONES																			
CASEIN HYDROLYSATE (ACID)	L41	<5	37.3	5.3	8.2	64	22	7.0	30.2	0.07	86	54	25	1	1.4	0.5	14	16	0.1
PEPTONISED MILK	L32	<5	7.9	1.8	5.3	34	28	6.5	1.6	2.0	11000	1196	49	3	0.3	1.9	4.0	42	<0.1
TRYPTONE	L42	<5	7.6	3.7	13.3	28	13	7.3	0.3	0.4	1350	200	54	2	0.6	28	1.7	28	0.1
TRYPTONE T	L43	<5	14.5	3.5	11.7	30	15	7.0	3.5	2.4	280	24	3	2	0.6	4.1	2.2	11	0.1
LACTALBUMIN HYDROLYSATE	L48	5.4	6.0	5.4	12.5	43	38	6.5	0.2	0.6	890	300	47	<1	0.1	1.3	1.5	4.1	<0.1
EXTRACTS																			
YEAST EXTRACT	L21	<5	13.3	5.3	10.9	49	ND	7.0	0.3	7.0	155	205	52	2	0.7	1.3	3.0	94	3.1
LAB-LEMCO	L29	<5	9.7	2.5	13.3	19	ND	7.2	1.1	1.9	140	140	20	2	0.3	0.4	9.8	18	0.5
MALT EXTRACT	L39	<5	1.2	0.6	1.1	55	ND	5.6	0.1	0.5	140	703	9	3	0.3	1.7	10.5	1.2	<0.1

ND = NOT DETERMINED

TABLE OF ANALYSIS

AMINO ACID ANALYSIS

TYPICAL AMINO ACIDS % W/W PEPTONE		ALANINE	ARGININE	ASPARTIC ACID	CYCTINE	GLUTAMIC ACID	GLYCINE	ISOLEUCINE	LEUCINE	LYSINE	METHIONINE	PHENYLALANINE	PROLINE	SERINE	THREONINE	TRYPTOPHAN	TYROSINE	VALINE
MEAT PEPTONES																		
LIVER DIGEST NEUTRALISED	L27	3.40	2.89	5.85	0.34	10.38	3.83	2.11	5.96	6.02	0.95	2.45	2.38	0.83	1.42	1.02	2.54	3.05
BACTERIOLOGICAL PEPTONE	L37	3.92	4.99	6.06	1.66	9.93	7.71	3.81	3.79	4.38	1.58	2.60	5.83	2.81	1.25	0.66	0.39	3.33
BACTERIOLOGICAL PEPTONE NEUTRALISED	L34	4.28	4.58	5.86	0.84	10.35	7.75	1.02	3.65	4.04	1.27	2.68	6.25	1.76	1.47	0.89	0.33	3.85
MYCOLOGICAL PEPTONE	L40	5.12	4.85	6.03	0.81	11.41	4.88	2.33	5.81	5.90	1.68	2.53	4.82	1.07	2.38	0.41	2.51	3.75
TRYPTOSE	L47	4.14	2.70	9.4	0.61	12.88	5.88	3.11	4.06	5.32	0.98	3.53	4.97	0.95	1.95	0.89	0.89	4.01
PEPTONE P	L49	6.75	4.32	7.93	0.42	11.51	9.78	2.41	3.70	4.86	1.48	2.98	5.80	1.24	2.41	0.50	0.99	3.71
SPECIAL PEPTONE	L72	5.54	3.95	6.40	0.33	14.99	2.28	3.11	4.88	5.59	2.7	3.36	7.11	1.30	2.02	1.35	1.84	3.89
PROTEOSE PEPTONE	L85	3.81	5.80	5.85	0.15	13.78	4.45	4.58	6.01	4.61	1.08	4.66	5.99	2.18	2.75	0.75	1.77	4.11
GELATIN PEPTONE	L92	6.31	7.75	4.68	0.12	9.13	20.95	1.66	2.99	3.89	0.96	1.71	12.10	3.61	1.99	0.25	0.82	2.09
VEGETABLE PEPTONE																		
SOYA PEPTONE	L44	2.57	4.64	7.06	0.53	14.71	2.83	2.51	4.31	3.77	0.62	0.38	3.40	0.67	1.68	0.64	2.09	3.65
CASEIN AND OTHER MILK DERIVED PEPTONES																		
CASEIN HYDROLYSATE (ACID)	L41	1.73	1.66	4.06	0.91	13.34	1.14	1.84	3.33	3.72	1.37	2.28	5.56	1.03	1.44	<0.1	1.57	3.38
PEPTONISED MILK	L32	1.98	0.75	2.71	0.44	9.63	1.94	1.20	3.5	2.62	0.93	1.78	4.01	1.53	1.37	0.53	1.21	2.89
TRYPTONE	L42	3.12	5.53	7.31	0.22	17.61	1.99	2.51	6.88	7.17	2.08	3.43	7.99	1.29	1.87	1.03	3.10	5.47
TRYPTONE T	L43	3.07	5.80	5.10	0.28	18.90	1.95	2.55	6.33	8.28	2.10	3.60	9.08	0.96	1.95	1.01	2.47	5.52
LACTALBUMIN HYDROLYSATE	L48	4.47	3.49	7.55	2.41	11.25	1.32	4.99	10.4	9.65	2.00	3.25	3.58	1.09	4.32	2.74	2.64	3.0
EXTRACTS																		
YEAST EXTRACT	L21	0.91	3.31	7.07	0.76	13.49	5.95	4.81	6.04	5.40	0.80	3.78	0.88	3.42	2.73	0.85	4.95	1.00
LAB-LEMCO	L29	5.85	7.10	5.10	0.68	10.71	10.85	3.17	3.15	4.78	2.61	2.34	7.79	1.87	2.54	0.34	0.66	3.06
MALT EXTRACT	L39	NOT DETERMINED														NOT DETERMINED		

OXOID AGARS

Agar is a complex mixture of polysaccharides extracted from species of the red algae known as agarophytes (*Gelidium*, *Gracilaria*, *Pterocladia*, *Acanthopeltis* and *Ahnfeltia* species). It is a sulphuric acid ester of a linear galactan, soluble in hot water but insoluble in cold water. A 1.5% w/v aqueous solution should set at 32–39°C and not melt below 85°C.

There are two dominating polysaccharides in agar which particularly affect its performance in culture media.

1 A virtually neutral polymer, agarose – (1–4) linked 3,6-anhydro-α-L-galactose alternating with (1–3) linked β-D-galactose.
2 A charged polymer, agaropectin, having the same repeating unit as agarose but with some of the 3,6-anhydro-L-galactose residues replaced with L-galactose sulphate residues, together with partial replacement of the D-galactose residues with pyruvic acid acetal 4,6-0-(1-carboxyethylidene)-D-galactose.

Agarose is the component responsible for the high-strength gelling properties of agar, whereas agaropectin provides the viscous properties. The proportion of agarose to agaropectin in agar varies according to the algae of origin but it can be as high as 75% agarose to 25% agaropectin.

The characteristic property of agar to form high-strength gels which are reversible with a hysteresis cycle over a range of 40°C is due to three equatorial hydrogen atoms on the 3,6-anhydro-L-galactose residues which constrain the molecule to form a helix with a threefold screw axis. It is the interaction of these helices which causes gel formation.

Agar is hydrolysed with heat at acid pH values because the 3,6-anhydro-α-L-galactoside linkage is very susceptible to acid cleavage.

Agar is manufactured in many parts of the world, although it is essential to locate the industry near suitable beds of algae and have efficient low-cost methods of harvesting the weed. It requires 100 tons of fresh water to produce one ton of dried agar, therefore the quality of the local water will influence the quality of the processed agar.

The presence of 'free' metal ions of Ca, Mg and Fe in agar which can react with phosphate salts in culture media to form insoluble precipitates or hazes is undesirable. Equally undesirable is the presence of chelating compounds which can bind these cations and make them unavailable to the organisms. Lowering the phosphate level of the culture medium to overcome its interaction with the metals usually results in poor growth-promoting properties. Compatibility tests between agar and the various culture media formulae are essential.

The agars used in such tests vary as follows:

1 **Bacteriological agar** Clear, colourless products in which the mineral/metal components may be reduced making them satisfactory for most formulae.
2 **Processed bacteriological agar** Clear, colourless products in which the mineral/metal components have been reduced to low levels, making them compatible with all formulations.

   A further advantage of chemical processing to reduce divalent cations is that it overcomes the antagonism of certain agars to amino-glycoside antimicrobials and tetracycline. It also considerably improves the diffusion of antimicrobials in the disc-diffusion assay.
3 **Technical grade agar** Less clear and colourless products in which the higher mineral/metal components may have advantages in certain low-phosphate formulations.

All such agars must be free from toxicity to micro-organisms and free from impurities such as non-agar gums, nitrogenous compounds, insoluble salts, free sugar compounds, dead micro-organisms and live thermophilic organisms.

The process of agar production has been fully described by Whistler[1], Chapman[2] and Bridson & Brecker[3] further details on the properties and testing of bacteriological agar can be found in Bridson[4].

References

1 Whistler R. L. (1973) *Industrial Gums, 2nd Edn., Academic Press, New York, pp. 29–48.*
2 Chapman V. J. (1970) *Seaweeds and their Uses. 2nd Edn., Methuen & Co. London. pp. 151–193.*
3 Bridson E. Y. and Brecker A. (1970) *Methods in Microbiology. Vol. 3A, Academic Press, London, pp. 257–266.*
4 Bridson E. Y. (1978) *Natural and Synthetic Culture Media for Bacteria. In: Handbook series in nutrition and food. Section G. Vol III. Ohio. CRC Press. 91–281.*

Oxoid supplies two grades of agar as Laboratory Preparations for inclusion in culture media: Agar Bacteriological (Agar No.1) Code L11 Agar Technical (Agar No.3) Code L13.

A third grade of specially processed agar is supplied for immuno-electrophoresis and gel diffusion studies: Purified Agar Code L28, although it can also be used in culture media.

Technical Information

Typical Batch Analysis	*L11*	*L13*	*L28*
Moisture	7.0%	12.0%	7.0%
Ash	2.0%	4.2%	3.6%
Acid insoluble ash	<0.1%	<0.1%	<0.1%
SO_4	0.9%	1.7%	0.7%
Total nitrogen	0.1%	0.1%	0.1%
Ca	100ppm	400ppm	100ppm
Mg	40ppm	100ppm	70ppm
Fe	–	–	10ppm
Precipitate with phosphate	None	Medium	None
Working strength	1.0%	1.2%	1.0%
M_r index	0.32	0.4	0.23

The Electroendosmosis value (m_r) of an agar gel is defined as the relative mobility of dextran to that of crystalline human albumin. The lower the figure the more suitable the agar for electrophoresis studies.

AGAR BACTERIOLOGICAL (AGAR NO.1)

Code: L11

A processed bacteriological agar of very high working gel strength (1% w/v) which has low Ca and Mg levels. It is compatible with all culture media and it enables broth and agar formulations of the same medium to have very similar metal values. This characteristic is especially valuable in antimicrobial MIC studies where differences in mineral/metal content can profoundly influence the results. It is also a highly satisfactory agar for antimicrobial diffusion studies (disc diffusion susceptibility tests) because its low mineral/metal content allows free diffusion of antimicrobial substances.

AGAR TECHNICAL (AGAR NO.3)

Code: L13

A technical grade, high working gel strength agar (1.2% w/v) suitable for purposes where clarity and compatibility are not of prime importance or where the high mineral/metal content has cultural advantages. A technical grade agar which conforms to the specifications of the U.S. Pharmacopoeia and APHA.

PURIFIED AGAR

Code: L28

An agar that has been extensively processed to give a low electroendosmosis factor (m_r) enabling the product to be used in electrophoresis studies without the high expense of using agarose preparations. It can also be used for bacteriological culture media where its special properties are required. An agar recommended for immuno-electrophoresis and gel diffusion studies.

AGAR TABLETS (OXOID AGAR NO.3)

Code: CM49

Description
Compressed tablets of a pre-weighed amount of agar which are especially useful for solidifying liquid media or for the preparation of semi-solid media. Oxoid Agar Tablets are standardised to produce a satisfactory gel when dissolved in 10ml of liquid. This eliminates batch to batch variation and the differences found between one type of agar and another. The use of these tablets obviates weighing and filtration thus leading to considerable time saving.

Technique
To produce a satisfactory gel for plate cultures and slopes, add 1 tablet to every 10ml liquid medium and autoclave at 121°C for 15 minutes. With very acid media it is advisable to use 2 tablets to every 10–15ml of medium. In order to prepare semi-solid agar use 1 tablet to every 100ml of liquid medium.

BILE, BILE SALTS AND DERIVATIVES

Bile is a product of the liver and its composition varies according to its animal source and its state of preservation. It contains bile pigments, bile acids in free and conjugate form, fatty acids, cholesterol, mucin, lecithin, inorganic salts, ethereal sulphates, glycuronic acids, urea and porphyrins. The role of the liver is to detoxify bile acids and it does this by conjugating them to glycine or taurine. A bile salt is the sodium salt of a conjugated bile acid.

A. BILE DESICCANT

Prepared from freshly collected animal bile (ox or sheep) filtered, concentrated at low temperatures and dried. When reconstituted it should form clear solutions with a brown-green colour at 10% w/v concentration. This was the first bile additive used as a selective agent in culture media by MacConkey in 1908.

Bile is a nutritive environment for a number of micro-organisms and putrefaction quickly occurs in the abattoir unless it is chilled or chemically preserved. The bacterial spoilage leads to deconjugation thus releasing varying amounts of free bile acids. As these acids are more toxic to bacteria than the conjugates there is considerable scope for variation in the selectivity of bile in culture media.

B. BILE SALTS

A further refinement in the use of bile is to precipitate the bile salts with hydrochloric acid and separate them from the bile pigments and other substances in crude bile.

Although this process yields bile salts, the product obtained may be a mixture of conjugates and free bile acids, depending on the quality of the bile used in the process.

The presence of more than 0.02% w/w desoxycholic acid in bile or bile salts indicates a degree of toxicity in the product which may make it unacceptable for certain media e.g. EE Broth CM317 and MacConkey Agar CM7.

Although bile-containing media are expected to suppress Gram-positive organisms and allow only bile-tolerant Gram-negative organisms to grow, some bile salts (free from bile acids) will allow staphylococci and streptococci to grow. Particular advantage is taken of this characteristic in the United Kingdom where MacConkey Agar CM7/CM7b is used as a general-purpose medium for clinical bacteriology. The growth of faecal streptococci and staphylococci would be looked for on this medium when it is used for the culture of urine, faeces and purulent material obtained from patients. The suppression of swarming growth of *Proteus mirabilis* in such specimens is a particular advantage of this medium.

Satisfactory bile salts are presented as a fine, white powder which, when reconstituted as a 2% w/v solution in distilled water, should be a clear, slightly yellow solution at pH 6.0. When incorporated into culture media, bile salts should not affect the colour of the indicator dyes or their subsequent change in colour. They should not form a surface scum or deposit in culture media.

C. BILE ACIDS

Bile acids are derived from deconjugated bile salts. Although micro-organisms can deconjugate bile compounds with great ease, the chemical process requires high temperatures for long periods at a high pH value. Hydrolysed bile acids are then precipitated with acid, washed, neutralised and dried. The predominant and most important bile acid is sodium desoxycholate which can be separated from the bile acid mixture and used as a relatively pure salt.

Leifson[1] showed that the constituent of bile which had greatest effect on bacterial growth was desoxycholic acid. He created desoxycholate-citrate agar when he discovered that the selectivity of sodium desoxycholate is enhanced when magnesium is removed from the medium. The role of sodium citrate in the medium is to chelate magnesium contributed by the other ingredients. It follows that the selectivity of the medium is lowered when magnesium is deliberately added to the formulation. This fact is important for the satisfactory growth of shigellae in desoxycholate media.

The increased selectivity of hydrolysed bile salts (Bile Salts No.3 or Bile Salts Mixture) is seen by the lowered concentration in culture media where it is added at 1.5g/l in place of 5g/l for normal bile salts. It is normal practice, however, to titrate the level into culture media by measuring the growth of appropriate test organisms as an indicator.

It can be seen from these variable factors that the production of standard bile-containing media is a difficult process which requires much skill and experience.

BILE SALTS

Code: L55

A standardised bile extract, consisting mainly of sodium glycocholate and sodium taurocholate, for use as a selective inhibitory agent in bacteriological culture media such as MacConkey Agar CM7 and MacConkey Broth CM5. Bile Salts L55 conforms to bacteriological requirements and batches are standardised, with respect to inhibitory properties, by the method of Burman[1]. It is generally employed in culture media at a concentration of 5 grams per litre. Bile Salts L55 meets the following specification:

Appearance – a 2% aqueous solution is light straw coloured, clear and free from extraneous matter.

Reaction – (2% aqueous solution) – pH 6 $\pm$ 0.2.

Minimal Effective Concentration (Burman[1]) – 0.25% – 0.5%.

Bacteriological Performance – MacConkey Broth and Agar made up with L55 at the minimal effective concentration (MEC) support satisfactory growth of bile-tolerant organisms including pathogenic

staphylococci. Gas production by clostridia in broths containing 0.5% of L55 is inhibited when the concentration is raised to 2%, as in Brilliant Green Bile (2%) Broth CM31 thus eliminating false positives in the 44°C test for *Escherichia coli*. During the testing of milk for *Escherichia coli* with Brilliant Green Bile (2%) Broth, false positives due to milk lactobacilli do not occur if the medium contains L55.

Reference

1 Burman N. P. (1955) *Proc. Soc. Water Treat. Exam. 4. 10.*

BILE SALTS NO.3

Code: L56

Oxoid Bile Salts No.3 was developed to meet the demand for a refined bile salt for use as a selective inhibitory agent in bacteriological culture media. It consists of a specially modified fraction of bile acid salts which is effective at less than one-third of the concentration of bile salts normally quoted. In selective media such as MacConkey Agar No.3, SS Agar and Violet Red Bile Agars the optimum concentration of Bile Salts No.3 is 0.15% w/v. In such media there is a very sharp differentiation between lactose-fermenters and non-lactose-fermenters of enteric origin – permitting the detection of scanty non-lactose-fermenters in the presence of numerous coliforms.

CHEMICALS FOR CULTURE MEDIA

The following ingredients for culture media are sufficiently defined to be classed as chemical reagents and would therefore be considered as reproducible. Nevertheless these products are subject to pre-shipment testing as part of a screening process and the accepted batches tested before release by the Quality laboratory.

Glucose Bacteriological	L71
Gelatin Bacteriological	L8
Haemoglobin powder soluble	L53
Lactose Bacteriological	L70
Skim milk powder	L31
Sodium biselenite	L121
Sodium chloride Bacteriological	L5
Sodium glutamate	L124
Sodium thioglycollate	L120

GLUCOSE (DEXTROSE)

Code: L71

A special bacteriological grade of anhydrous glucose for use in culture media. Each batch is tested chromatographically to ensure purity and correct identity.

GELATIN

Code: L8

Gelatin is a collagenous protein used for the solidification of culture media and for the detection and differentiation of certain proteolytic bacteria. Oxoid-Gelatin is a bacteriological grade which has been manufactured and selected specifically for use in culture media. It is readily soluble in water to give a clear solution, free from sulphite and other preservatives. A satisfactory firm gel is obtained from a 15% solution, and media containing this proportion of gelatin will withstand short-term autoclaving for 15 minutes at 121°C without significant loss of gel strength.

SOLUBLE HAEMOGLOBIN POWDER

Code: L53

Prepare a 2% w/v solution of Soluble Haemoglobin Powder L53 by adding 250ml of distilled water at 50°C to 5g of Haemoglobin Powder. Continually stir the solution during the addition of water. Sterilise by autoclaving at 121°C for 15 minutes.

LACTOSE BACTERIOLOGICAL

Code: L70

A special grade for inclusion in microbiological media. Each batch is tested chromatographically to ensure purity and correct identity.

SKIM MILK POWDER

Code: L31

The use of ordinary skim milk powder is undesirable in bacteriological media because of the presence of heat-resistant organisms which give rise to erroneous cultural results. Oxoid Skim Milk Powder is a special bacteriological grade of spray-dried skim milk free from thermophilic organisms.

Average analysis:

Moisture	5.0%
Ash	8.0%
Total Nitrogen	5.3%
Reducing Sugars (as lactose monohydrate)	48.0%
Ether Soluble Extract	0.25%

Mix the powder to a smooth paste with a small quantity of distilled water, then gradually add more distilled water until a 10% w/v mixture is obtained. This is equivalent to fresh milk, and may be sterilised by autoclaving for 5 minutes at 121°C. Care should be taken not to overheat during sterilisation, otherwise caramelisation will occur. This product may be used alone or as a constituent of more complex bacteriological culture media. A 10% 'solution' of Skim Milk Powder, containing 0.001% of bromo-cresol purple forms a highly satisfactory purple milk which may be employed for the cultivation of dairy organisms or for the differentiation of *Clostridium* species, etc. Media containing skim milk powder are of particular value for diagnostic cultural tests involving the fermentation of lactose and digestion or coagulation of casein.

This product is not always free from antimicrobial residues. Where antibiotic-free milk powder is specified in a formulation, tests must be carried out to determine if it is satisfactory.

SODIUM BISELENITE
(Sodium hydrogen selenite)

Code: L121

For use in Oxoid Selenite Broth Base CM395/CM396 and Mannitol Selenite Broth Base CM399.
CAP SECURELY AFTER USE.

Dissolve 4 grams in 1 litre of distilled water and use this solution to reconstitute the base medium or tablets.

Toxic by inhalation and if swallowed. Danger of cumulative effects.

SODIUM CHLORIDE

Code: L5

See also Saline Tablets BR53.

This product is prepared from analytical grade salt to avoid problems associated with additives.

SODIUM GLUTAMATE

Code: L124

For use with Minerals Modified Medium Base (CM607).

SODIUM THIOGLYCOLLATE

Code: L120

For use in bacteriological culture media in order to lower their oxidation-reduction potential and to neutralise mercurial preservatives.

IRRITANT

Irritating to eyes, respiratory system and skin.

Harmful in contact with skin and if swallowed.

4
SELECTIVE SUPPLEMENTS, STERILE REAGENTS, PREPARED MEDIA & DIP SLIDES

November 1998

SELECTIVE SUPPLEMENTS AND STERILE REAGENTS

SELECTIVE SUPPLEMENTS

Code: SR

Selective Supplements are freeze-dried, accurate preparations of antimicrobials which are added, normally one vial per 500ml of sterile cooled medium, to base nutrient media to create specific, selective media.

The concepts of antimicrobial selective microbiology are now widely accepted and have proved their value, particularly in clinical and food microbiological laboratories[1].

A result of the use of these selective supplements is that it is possible for non-specialised laboratories to isolate unusual but important organisms from contaminated clinical samples or food raw materials. *Legionella*, *Listeria*, *Campylobacter*, *Yersinia*, *Brucella* and *Bordetella* species as well as many other 'difficult' organisms can be isolated and confident statements made about their presence or absence in the material examined. Whilst the specialised laboratory can often find their use makes the isolation procedure very much simpler than previous methods.

New and improved antimicrobial mixtures continue to be published and will be made available to laboratories as soon as field trials have proved the value of the formulation.

Selection does not lie solely in the antimicrobial supplement, it is often a combination of specific nutrient base with the supplement. Thus specified base media formulations are paired with specific selective supplements and the results can be predicted. More difficulty can be expected where selective supplements are added to unspecified base media because some changes in media constituents can strongly influence the selectivity of the antimicrobials.

The concentration of the antimicrobial mixture in the supplement is based on the published papers of workers who have tested many strains of the selected organisms and who have measured their MIC values to ensure lack of inhibition. However, across the world there exists the possibility that strains in other countries may have MIC values that are different.

If there is doubt about the inhibitory properties of selective media on local isolates then tests should be set up to make comparative quantitative assessments on selective and non-selective media. The base nutrient medium can be prepared with and without selective supplement. The local strains can then be inoculated on the media in a quantitative way i.e. measured drop inocula. After incubation a comparative count is made. There should be less than one log number difference between the counts on the two media. If a greater reduction in growth occurs on the selective medium then the volume of anti-microbial solution should be reduced and tested again. Where a reduction in volume of the supplement occurs it would be useful to test a mixed culture of common contaminants to ensure that the altered level is still selective.

An example of a change in concentration of antimicrobials can be seen in the description of Clostridium difficile Selective Medium CM601 page 77. In this particular case a change in specimen processing i.e. an alcohol-shock treatment, helped reduce contamination but appeared to make the clostridia more susceptible to full strength antimicrobials. The facility to alter the concentrations of the selective antimicrobials in the medium is one of the many advantages in using freeze-dried antimicrobial supplements.

Full details of the use of the Selective Supplements are given in Section 2 Culture Media. The details given in this section cover the contents and the reconstitution of the available supplements with directions to the appropriate part of Section 2.

Reference

1 Bridson E. Y. (1982) *Selective Culture Media – a renewed activity. Med. Lab. Sci. 39. 1–2.*

GENERAL GUIDELINES TO THE PREPARATION OF SELECTIVE CULTURE MEDIA

The considerations that apply to the preparation of general purpose media require emphasis when applied to Selective culture media.

For optimal performance the medium must be sensitive enough to detect the presence of very small numbers of organisms which may have been stressed or actually damaged. Different strains of organisms vary in their resistance to selective agents and in some cases the concentration of an agent may exceed or be close to a level that a very sensitive strain will not tolerate. In such cases it is apparent that a distorted view of the presence and numbers of a given organism could be obtained. For this reason media must be chosen rationally to match their purpose and the user should be familiar with the limitations of the different formulations under the conditions of use.

The various components of a selective medium must be matched for best performance. For this reason the selective agent and the base must be considered as a compatible 'set'. The combination of supplement and base ensures that the end performance of the medium will meet the specification demanded. It is well known that identical quantities of selective substances will perform differently in different nutrient bases. This is because their disruptive activity in the organism's metabolism is heavily influenced by the presence of growth factors, mineral protective substances, pH, etc.

Careful storage of dehydrated media is very important and it does not always receive the attention it deserves. Manufacturers' instructions regarding storage in a cool, dry, dark place should be carefully observed and once a container has been opened the contents should be used as soon as possible. Moisture must be kept out. If not, the more hygroscopic media may change in weight, and thus alter the

concentration of the components in the final medium and – more serious still – actual physical deterioration may occur, particularly if storage is in warm conditions. The effect of this on performance of selective media would be considerable.

Some selective agents are photo-sensitive and although storage should be in the dark the problem is probably of greater importance when the medium has been reconstituted ready for use.

For best performance, preparation of the selective medium needs careful attention to the following points:

1 Use only completely clean equipment. Residues from incomplete cleaning can have a significant effect.

   Care must always be taken to prevent cross contamination of one medium with another by dirty spatulae, balance pans, pipettes, etc.

2 The choice of water is important. Glass distilled water is probably best, but de-ionised water is satisfactory. It should be remembered, however, that de-ionisers may harbour microbial flora, some of which may produce inhibitory substances. Tap water should not be used.

3 Weighing and measurement must be precise otherwise pH and concentrations of the constituents will be incorrect.

4 Finely powdered products can be scattered as dust and some selective agents could become a health hazard if inhaled. Selective agents presented in vials overcome this objection as the reconstitution is done by adding the sterile distilled water or other solvent before the cap is removed.

5 The medium must be well mixed to ensure even distribution, particularly of selective agents which may be present in very small amounts. The addition of a solution of the selective agent to the basal medium makes proper distribution more certain.

6 Carry out the sterilisation procedure meticulously, taking care not to overheat. In most cases a better medium will be obtained if the selective agent is added aseptically after sterilisation.

7 Selective agents, including antibiotics, may be labile especially in solution. Antibiotics are best kept dry until the time of use. Limited shelf life, unknown potency and cross-contamination are all special difficulties associated with antibiotics.

8 Media should be used as soon as possible after preparation, but if storage is needed this should be in cool, moist and dark conditions to keep changes in the medium to a minimum.

It was consideration of the special requirements of selective media detailed above that led to the conception of the Oxoid range of freeze-dried supplements, which have been carefully standardised for use with specified Oxoid dehydrated media. This has not only greatly increased convenience for the user, but has virtually eliminated health hazards to the laboratory preparations staff.

Oxoid Selective Media have the following features

1 Labile and hazardous components are presented in nitrogen-flushed glass vials.

2 The stability of critical components is assured. A long shelf life (up to two years in most cases) is guaranteed.

3 Each vial contains a measured dose sufficient for a standard volume of medium (usually 500ml). It is easy to reconstitute.

4 The performance of the medium made with the selected basal medium and its compatible supplement will be reproducible.

5 Because the selective agents are not held for long periods as solutions, but reconstituted immediately before use, they always have optimal activity.

6 A futher advantage of this concept is that some flexibility of concentration is available. Although the vials are designed to be emptied into standard volumes of medium, there is individual choice of using less or more of the selective agent. In some circumstances there may be advantages in changing the concentration of the selective agent in order to yield optimum recovery of the desired organism and suppression of other flora.

The benefits of using the Oxoid range of Selective Media

These are evident from features described above, but they may be summed up as follows:

1 More reliable media with greatly reduced likelihood that labile ingredients will have deteriorated.

2 The work of the media preparation staff is made safer and easier.

3 The microbiologist is free to concentrate on end product performance, confident of obtaining reproducible results.

SELECTIVE MICROBIOLOGY

Oxoid freeze-dried, selective and growth supplements are used to prepare selective, differential or enriched culture media. They are designed to give optimum performance when added to the appropriate Oxoid base media shown in the directions.

All supplements should be stored at 2–8°C.

NOTE: before opening supplements containing cycloheximide read the comments under HAZARDS page 2-7.

AMPICILLIN SELECTIVE SUPPLEMENT

Code: SR136

A selective supplement for the isolation of A. hydrophila when used with Aeromonas Medium Base (Ryan) CM833. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Ampicillin	2.5mg

Directions
To one vial add 2ml of sterile distilled water and dissolve the contents completely. Add the contents aseptically to 500ml of Aeromonas Agar Base (Ryan) CM833 cooled to 50°C. Mix well and pour into petri dishes.

ANAEROBE SELECTIVE SUPPLEMENT G-N

Code: SR108

A selective supplement for the isolation of Gram-negative anaerobes when used with Wilkins-Chalgren Agar CM619. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Haemin	2.5mg
Menadione	0.25mg
Sodium succinate	1.25g
Nalidixic acid	5.0mg
Vancomycin	1.25mg

Directions
To one vial aseptically add 10ml of sterile distilled water and mix gently to dissolve completely. Avoid frothing. Add the contents to 500ml of sterile Wilkins-Chalgren Medium, prepared from Wilkins-Chalgren Anaerobe Agar CM619 plus 5% (v/v) defibrinated horse blood SR50 or SR51. **Do not add 'Tween 80'**. Mix gently and pour into sterile petri dishes.

ANAEROBE SELECTIVE SUPPLEMENT N-S

Code: SR107

A selective supplement for the isolation of Non-Sporing anaerobes when used with Wilkins-Chalgren Agar CM 619. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Haemin	2.5mg
Menadione	0.25mg
Sodium pyruvate	500.0mg
Nalidixic acid	5.0mg

Directions
To one vial aseptically add 10ml of sterile distilled water and mix gently to disolve completely. Avoid frothing. Add the contents to 500ml of sterile Wilkins-Chalgren Medium, prepared from Wilkins-Chalgren Anaerobe Agar CM619 plus 0.1% (v/v) 'Tween 80' and 5% (v/v) defibrinated horse blood SR50 or SR51. Mix gently and pour into sterile petri dishes.

BACILLUS CEREUS SELECTIVE SUPPLEMENT

Code: SR99

A selective supplement for the isolation and enumeration of B. cereus when used with Bacillus cereus Selective Agar CM617. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Polymyxin B	50,000 IU

Directions
To one vial aseptically add 2ml of sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Add to this 475ml of sterile molten Bacillus cereus Agar Base CM617 cooled to 50°C, together with 25ml of sterile Egg Yolk Emulsion SR47. Mix well and pour into sterile petri dishes.

BORDETELLA SELECTIVE SUPPLEMENT

Code: SR82

A selective supplement for the isolation of Bordetella species when used with Bordet-Gengou Agar Base CM267 or Charcoal Agar CM119. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Cephalexin	20mg

Directions
To one vial add 2ml of sterile distilled water and dissolve the powder completely. Add the contents aseptically to 500ml of sterile, molten Charcoal Agar CM119, cooled to 50°C, together with 10% defibrinated horse blood SR50. Mix well before pouring into sterile petri dishes.

Bordet-Gengou Agar CM267 may be used instead of Charcoal Agar. Add the reconstituted contents of one vial to 500ml of Bordet-Gengou Agar cooled to 50°C, to which 75–100ml of fresh defibrinated horse blood SR50 has been added.

The vial contents may also be added to half strength Charcoal Agar plus 10% defibrinated horse blood (SR50), for use as a transport medium for *Bordetella pertussis.*

BRUCELLA SELECTIVE SUPPLEMENT

Code: SR83

A selective supplement for the isolation of Brucella species when used with Blood Agar Base No.2 CM271, Columbia Agar CM331 or Brucella Agar Bases CM169 or CM691. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Polymixin B	2,500 IU
Bacitracin	12,500 IU
Cycloheximide	50mg
Nalidixic acid	2.5mg
Nystatin	50,000 IU
Vancomycin	10mg

Directions
Add 10ml of a 50:50 mixture of methanol and sterile distilled water to form a suspension. Incubate for 10–15 minutes at 37°C. Mix thoroughly and immediately add the vial contents to 500ml of sterile nutrient medium cooled to 50°C prepared from Brucella Medium Base CM169 or CM691, Columbia Agar Base CM331 or Blood Agar Base No.2 CM271 together with 5–10% v/v inactivated horse serum SR35 and 1–5% w/v sterile solution of glucose. Mix well and pour into sterile petri dishes.

CAMPYLOBACTER GROWTH SUPPLEMENT

Code: SR84

Supplement for the enhanced growth and aerotolerance of campylobacter.

Vial contents (each vial is sufficient for 500ml of medium)

Sodium pyruvate	0.125g
Sodium metabisulphite	0.125g
Ferrous sulphate (hydrated salt)	0.125g

Directions
To rehydrate the contents of the vial, aseptically add 2ml of sterile distilled water and invert to dissolve. Avoid frothing of solution.

Add the contents of one vial to 500ml of a sterile nutrient medium cooled to 50–55°C prepared from Oxoid Columbia Agar CM331, Blood Agar Base No.2 CM271, or Campylobacter Agar Base CM689, with 5–7% lysed defibrinated horse or sheep blood, and the rehydrated contents of one vial of Campylobacter Antibiotic Supplement SR69, SR85 or SR98. Mix gently and pour aseptically into sterile petri dishes.

CAMPYLOBACTER SELECTIVE SUPPLEMENT (BLASER-WANG)

Code: SR98

A selective supplement for the isolation of campylobacters when used with Blood Agar Base No.2 CM271, Columbia Agar CM331 or Brucella Agar Bases CM169 or CM691. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Vancomycin	5mg
Polymixin B	1,250 IU
Trimethoprim	2.5mg
Amphotericin B	1.0mg
Cephalothin	7.5mg

Directions
To rehydrate the contents of the vial, aseptically add 2ml of sterile distilled water and mix gently to dissolve. Avoid frothing. Add the contents of one vial to 500ml of sterile nutrient medium cooled to 50–55°C prepared from Brucella Agar Base CM169 or CM691, Columbia Agar CM331 or Blood Agar Base No.2 CM271, with 10% sheep blood or 5–7% laked horse blood SR48. Mix gently and pour into sterile petri dishes.

CAMPYLOBACTER SELECTIVE SUPPLEMENT (BUTZLER)

Code: SR85

A selective supplement for the isolation of campylobacters at 35°C as well as 43°C when used with Blood Agar Base No.2 CM271, Columbia Agar CM331 or Brucella Agar Base CM169 or CM691. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Bacitracin	12,500 IU
Cycloheximide	25mg
Colistin sulphate	5,000 IU
Cephazolin sodium	7.5mg
Novobiocin	2.5mg

Directions
To the contents of one vial aseptically add 3ml of 50:50 ethanol/water and invert gently to dissolve. Avoid causing frothing. Add the contents of 1 vial to 500ml of sterile blood agar cooled to 50–55°C prepared from Columbia Agar CM331, Blood Agar Base No.2 CM169 or CM691 with 5–7% defibrinated horse blood. Mix well and pour into sterile petri dishes.

CAMPYLOBACTER SELECTIVE SUPPLEMENT (PRESTON)

Code: SR117

A selective supplement for the isolation of campylobacters when used with Campylobacter Agar Base CM689 or Nutrient Broth Base No.2 CM67. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Polymixin B	2,500 IU
Rifampicin	5mg
Trimethoprim	5mg
Cycloheximide	50mg

Directions
To reconstitute add 2ml of 50:50 acetone/sterile distilled water and mix gently to dissolve completely. Avoid frothing. Add the vial contents to 500ml of sterile basal medium prepared from Campylobacter Agar Base CM689 and 5% v/v Lysed Horse Blood SR48 or to 500ml of Enrichment Broth prepared from Nutrient Broth No.2 CM67, 5% v/v Lysed Horse Blood SR48 and Campylobacter Growth Supplement SR84.

CAMPYLOBACTER SELECTIVE SUPPLEMENT (SKIRROW)

Code: SR69

A selective supplement for the isolation of campylobacters when used with Blood Agar Base No.2 CM271, Columbia Agar CM331 or Brucella Agar Bases CM169 or CM691. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Vancomycin	5mg
Trimethoprim	2.5mg
Polymyxin B	1250 IU

Directions
To rehydrate the contents of the vial, aseptically add 2ml of sterile distilled water and mix gently to dissolve. Avoid frothing. Add the contents of one vial to 500ml of a sterile nutrient medium cooled to 50–55°C prepared from Brucella Agar Base CM169 or CM691, Columbia Agar CM331 or Blood Agar Base No.2 CM271, with 10% sheep blood or 5–7% laked horse blood SR48. Mix gently and pour into sterile petri dishes.

CCDA SELECTIVE SUPPLEMENT

Code: SR155

An improved selective supplement for Campylobacter Blood Free Selective Agar CM739. See Section 2.

Vial contents (each vial is sufficient to supplement 500ml of medium)

Cefoperazone	16mg
Amphotericin B	5mg

Directions
To one vial add 2ml of sterile distilled water and mix gently to dissolve completely. Avoid frothing. Add the contents to 500ml of sterile Blood Free Campylobacter Selective Agar Base CM739 cooled to 50°C. Mix well and pour into sterile petri dishes.

CAMPYLOBACTER SELECTIVE SUPPLEMENT (KARMALI)

Code: SR167

Vial contents:

Haemin	16mg	(equivalent to 32mg/l)
Sodium pyruvate	50mg	(equivalent to 100mg/l)
Cefoperazone	16mg	(equivalent to 32mg/l)
Vancomycin	10mg	(equivalent to 20mg/l)
Cycloheximide	50mg	(equivalent to 100mg/l)

Directions
Add 21.5 grams of Campylobacter Agar Base (Karmali) CM935 to 500mls of distilled water and bring to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Aseptically add 1 vial of Campylobacter Selective Supplement (Karmali) SR167 reconstituted with 2ml of sterile distilled water. Mix well and pour into sterile petri dishes.

Description
Campylobacter Medium (Karmali) is based on the formulation described by Karmali et al[1] and is recommended for the isolation of *Campylobacter jejuni* and *Campylobacter coli* from clinical specimens.

CEFOPERAZONE, AMPHOTERICIN B, TEICOPLANIN SUPPLEMENT (CAT)

Code: SR174

A selective supplement for the isolation of thermophilic Campylobacter spp. and improved recovery of Campylobacter upsaliensis from faeces.

Vial contents

CAT Supplement	*Milligrams per litre*
Cefoperazone	8.0
Teicoplanin	4.0
Amphotericin B	10.0

Directions
Aseptically add 4ml of sterile distilled water to the vial. Mix gently to resuspend the supplement. Prepare 500ml of sterile Blood Free Campylobacter Agar Base CM739 as directed. Cool to 50°C and aseptically add one vial of SR174E reconstituted as directed above. Mix well and pour the resulting CAT medium into sterile petri dishes. Incubate cultures at 37°C for 48–72 hours in a microaerobic atmosphere.

C-F-C SELECTIVE SUPPLEMENT

Code: SR103

A selective supplement for the isolation of low temperature pseudomonads when used with Pseudomonas Agar Base CM559. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Cetrimide	5.0mg
Fucidin	5.0mg
Cephaloridine	25.0mg

Directions
To one vial aseptically add 2ml of sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Add the contents to 500ml of sterile Pseudomonas basal medium, cooled to 50–55°C, prepared from Pseudomonas Agar Base CM559 plus 5ml of glycerol.

C-N SELECTIVE SUPPLEMENT

Code: SR102

A selective supplement for the isolation of Ps. aeruginosa when used with Pseudomonas Agar Base CM559. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Cetrimide	100mg
Sodium nalidixate	7.5mg

Directions
To one vial aseptically add 2ml of sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Add the contents to 500ml of sterile Pseudomonas basal medium, cooled to 50–55°C, prepared from Pseudomonas Agar Base CM559 plus 5ml of glycerol.

CHLORAMPHENICOL SELECTIVE SUPPLEMENT

Code: SR78

A selective supplement for the isolation of yeasts and moulds when used with Rose-Bengal Chloramphenicol Agar CM549, DRBC Agar Base CM727 or Dichloran-Glycerol (DG18) Agar Base CM729. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Chloramphenicol	50mg

Directions
To one vial add 3ml of acetone and dissolve the powder completely. Add the contents to 500ml of Rose-Bengal Chloramphenicol Agar Base CM549 or other media requiring addition of chloramphenicol. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Mix gently and pour into sterile petri dishes.

CLOSTRIDIUM DIFFICILE SELECTIVE SUPPLEMENT

Code: SR96

A selective supplement for the isolation of C. difficile when used with Clostridium Difficile Agar Base CM601. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

D-cycloserine	250mg
Cefoxitin	8mg

Directions
To one vial aseptically add 2ml of sterile distilled water and mix gently to dissolve the contents completely. Add to 500ml of sterile molten Clostridium Difficile Agar Base CM601 cooled to 50°C together with 7% v/v Defibrinated Horse Blood SR50. Mix well and pour into sterile petri dishes.

CLOSTRIDIUM DIFFICILE MOXALACTAM NORFLOXACIN (CDMN) SELECTIVE SUPPLEMENT

Code: SR173

An alternative more selective supplement.

Vial contents

CDNM Supplement SR173	*Milligrams per litre*
Cysteine hydrochloride	500.0
Norfloxacin	12.0
Moxalactam	32.0

Directions
Aseptically add 2ml of sterile distilled water to each vial of supplement and mix gently to dissolve completely. Avoid frothing. Prepare 500ml of sterile Clostridium difficile Agar Base CM601 as directed. Cool to 50°C and aseptically add 7% (v/v) of Defibrinated Horse Blood SR50 and the contents of one vial of SR173E (reconstituted as directed above). Mix well and pour the resulting CDMN Agar into sterile petri dishes.

CEFIXIME–TELLURITE SUPPLEMENT

Code: SR172

A freeze-dried supplement for use with Sorbitol MacConkey Agar, CM813, for the selective isolation of E. coli O157:H7.

Vial contents:

	Milligrams per litre
Potassium tellurite	2.5
Cefixime	0.05

Directions
Aseptically add 2ml of sterile distilled water to 1 vial of Cefixime-Tellurite Supplement SR172E. Mix gently to dissolve the contents completely. Add the vial contents to 500ml of Sorbitol MacConkey Agar prepared as directed and cooled to 50°C. Mix well and pour the medium into petri dishes.

DERMASEL SELECTIVE SUPPLEMENT

Code: SR75

A selective supplement for the isolation of common dermatophyte fungi when used with Dermasel Agar CM539. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Cycloheximide	200mg
Chloramphenicol	25mg

Directions
To one vial add 3ml of acetone and mix gently to dissolve completely. Add to 500ml of Dermasel Agar CM539. Mix gently and sterilise by autoclaving at 121°C for 10 minutes. Avoid overheating at any time.

FRASER SUPPLEMENT

Code: SR156

A selective supplement for the isolation of Listeria spp. when used with Fraser Broth CM895. See Section 2.

Vial contents (each vial is sufficient to supplement 500ml of medium)

Ferric ammonium citrate	0.25g
Nalidixic acid	10.0mg
Acriflavine hydrochloride	12.5mg

Directions
To one vial add 5ml of ethanol/sterile distilled water (1:1) and mix gently to dissolve. Add the contents to 500ml of sterile Fraser Broth Base CM895 cooled to 50°C. Mix well and distribute into sterile containers.

HALF FRASER SUPPLEMENT

Code: SR166E

A selective supplement for the isolation of Listeria spp. from food and environmental samples when used with Fraser Broth CM895.

Vial contents (each vial is sufficient to supplement 225ml of medium)

Ferric ammonium citrate	112.50mg
Nalidixic acid	2.25mg
Acriflavine hydrochloride	2.8125mg

Directions
Suspend 14.35 grams of Fraser Broth (CM895) in 225ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of Half Fraser Selective Supplement (SR166E) reconstituted with 4ml of (1:1) ethanol/sterile distilled water and mixed gently to dissolve. Mix well and distribute into final containers.

GARDNERELLA VAGINALIS SELECTIVE SUPPLEMENT

Code: SR119

A selective supplement for the isolation of G. vaginalis when used with Columbia Agar Base CM331. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Gentamicin sulphate	2.0mg
Nalidixic acid	15mg
Amphotericin B	1.0mg

Directions
To reconstitute add 2ml of ethanol/sterile distilled water (1:1) to one vial. Add aseptically the contents of one vial to 450ml of sterile Columbia Blood Agar Base CM331 cooled to 50°C, and supplement with 50ml of sterile human, rabbit or horse blood. Mix well. For the surface inoculation technique pour into sterile petri dishes and for the double layer technique place the medium in a water bath at 50°C.

GC SELECTIVE SUPPLEMENT

Code: SR56

A selective supplement for the isolation of pathogenic neisseria when used with GC Agar Base CM367. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Yeast autolysate	5.0g
Glucose	0.75g
Sodium bicarbonate	0.075g
Vancomycin	1.5mg
Colistin methane sulphonate	3.75mg
Nystatin	6,250 IU
Trimethoprim	2.5mg

Directions
To one vial add 15ml of sterile distilled water (previously warmed to 37°C to 45°C) and shake vigorously until the contents are completely dissolved. Add the contents to 485ml of sterile Thayer-Martin Medium, cooled to 50–55°C, prepared from Oxoid GC Agar Base CM367 and 2% (w/v) Soluble Haemoglobin Powder L53.

HELICOBACTER PYLORI SELECTIVE SUPPLEMENT (DENT)

Code: SR147

A selective supplement for the isolation of H. pylori from clinical specimens.

Vial contents (each vial is sufficient for 500ml of medium)

Vancomycin	5.0mg
Trimethoprim	2.5mg
Cefsulodin	2.5mg
Amphotericin B	2.5mg

Directions
To one vial add 2ml of sterile distilled water and dissolve the powder completely. Avoid frothing. Add the contents aseptically to 500ml of sterile Columbia Blood Agar Base CM331 at 50°C. Add 35ml of Laked Horse Blood SR48 and mix well before pouring into sterile petri dishes.

KANAMYCIN SULPHATE SELECTIVE SUPPLEMENT

Code: SR92

A selective supplement for the isolation of enterococci when used with Kanamycin Aesculin Azide Agar Base CM591 or Kanamycin Aesculin Broth Base CM771. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Kanamycin sulphate	10mg

Directions
Add 2ml of sterile distilled water to a vial and mix gently to dissolve completely. Add the contents to 500ml of either Kanamycin Azide Agar Base CM591 or Kanamycin Azide Broth Base CM771.

LCAT SELECTIVE SUPPLEMENT

Code: SR95

A selective supplement for the isolation of pathogenic neisseria when use with GC Agar Base CM367. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Lincomycin	0.5mg
Colistin sulphate	3.0mg
Amphotericin B	0.5mg
Trimethoprim	3.25mg

Directions
To one vial aseptically add 10ml of sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Add the contents to 490ml of sterile GC Agar Base CM367, cooled to 50–55°C, containing 50ml Defibrinated Horse Blood (SR50) lysed with 0.5% (v/v) saponin, and the rehydrated contents of one vial of Yeast Autolysate Supplement SR105.

LEGIONELLA BCYE GROWTH SUPPLEMENTS

Code: SR110

A growth supplement for the isolation of legionellae when used with Legionella CYE Agar Base CM655. See Section 2.

Vial contents (each vial is sufficient for 100ml of medium)

ACES Buffer/Potassium hydroxide	1.0g
Ferric pyrophosphate	0.025g
L-cysteine HCl	0.04g
α-ketoglutarate	0.1g

Directions
To one vial aseptically add 10ml of warm sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Add the contents to 90ml of sterile Legionella CYE Agar Base CM655 cooled to 50–55°C. Mix gently and pour into sterile petri dishes.

Code: SR175

Supplement omitting L-cysteine, for presumptive identification of Legionella spp.

Vial contents (each vial is sufficient for 100ml of medium)

	grams per litre
ACES Buffer/Potassium hydroxide	10
Ferric pyrophosphate	0.25
L-cysteine hydrochloride	Nil
α-ketoglutarate	1.0

Directions
Prepare in exactly the same way as for SR110 above.

LEGIONELLA BMPA SELECTIVE SUPPLEMENT

Code: SR111

A selective supplement for the isolation of legionellae when used with Legionella BCYE Growth Supplement SR110 and Legionella CYE Agar Base CM655. See Section 2.

Vial contents (each vial is sufficient for 100ml of medium)

Cefamandole	400μg
Polymyxin B	8,000 IU
Anisomycin	8mg

Directions
To one vial add 2ml of sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Aseptically add the vial contents to 100ml of BCYE-α Medium, cooled to 50°C, prepared from Legionella CYE Agar Base CM655 and Legionella BCYE-α Supplement SR110. Mix well and pour into sterile petri dishes.

LEGIONELLA (GVPC) SELECTIVE SUPPLEMENT

Code: SR152

A freeze-dried selective supplement for the isolation of Legionella spp. from environmental water samples, when used with Legionella CYE Agar Base CM655 and Legionella BCYE-α Growth Supplement SR110. See Section 2.

Vial contents (each vial is sufficient to supplement 500ml of BCYE-α Medium)

Glycine (Ammonia free)	1.5g
Vancomycin hydrochloride	0.5mg
Polymyxin B sulphate	39600 IU
Cycloheximide	40.0mg

Directions
To one vial aseptically add 10ml of sterile distilled water and mix gently to dissolve completely. Add the contents to 500ml of sterile BCYE-α Medium (prepared using Legionella CYE Agar Base CM655 to which one vial of Legionella BCYE-α Growth Supplement SR110C has been added), cooled to 50–55°C. Mix gently and pour into sterile petri dishes.

LEGIONELLA MWY SELECTIVE SUPPLEMENT

Code: SR118

A selective supplement for the isolation of legionellae when used with Legionella BCYE Growth Supplement SR110 and Legionella CYE Agar Base CM655. See Section 2.

Vial contents (each vial is sufficient for 100ml of medium)

Glycine	0.3g
Polymyxin B	5,000 IU
Anisomycin	8mg
Vancomycin	100μg
Bromothymol blue	1mg
Bromocresol purple	1mg

Directions
To one vial add 2ml of sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Aseptically add the vial contents to 100ml of BCYE-α Medium, cooled to 50°C, prepared from Legionella CYE Agar Base CM655 and Legionella BCYE-α Supplement SR110. Mix well and pour into sterile petri dishes.

LISTERIA PRIMARY SELECTIVE ENRICHMENT SUPPLEMENT (UVM I)

Code: SR142

A primary selective enrichment supplement for the isolation of listeria when used with Listeria Enrichment Broth Base (UVM Formulation) CM863. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Nalidixic acid	10.0mg
Acriflavine	6.0mg

Directions
To one vial add 2ml of sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Aseptically add the vial contents to 500ml of sterile Listeria Enrichment Broth Base (UVM Formulation) CM863, below 50°C. Mix well and pour required volumes into sterile containers.

LISTERIA SECONDARY SELECTIVE ENRICHMENT SUPPLEMENT (UVM II)

Code: SR143

A primary selective enrichment supplement for the isolation of listeria when used with Listeria Enrichment Broth Base (UVM Formulation) CM863. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Nalidixic acid	10.0mg
Acriflavine	12.5mg

Directions
To one vial add 2ml of sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Aseptically add the vial contents to 500ml of sterile Listeria Enrichment Broth Base (UVM Formulation) CM863, below 50°C. Mix well and pour required volumes into sterile containers.

LISTERIA SELECTIVE ENRICHMENT SUPPLEMENT

Code: SR141

A selective enrichment supplement for the isolation of listeria when used with Listeria Enrichment Broth Base CM862. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Nalidixic acid	20.0mg
Cycloheximide	25.0mg
Acriflavine	7.5mg

Directions
To one vial add 2ml of sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Aseptically add the vial contents to 500ml of sterile Listeria Enrichment Broth Base CM862, below 50°C. Mix well and pour required volumes into sterile containers.

LISTERIA SELECTIVE SUPPLEMENT (OXFORD)

Code: SR140

A selective supplement for the isolation of listeria when used with Listeria Selective Agar Base (Oxford Formulation) CM856. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Cycloheximide	200mg
Colistin sulphate	10mg
Acriflavine	2.5mg
Cefotetan	1.0mg
Fosfomycin	5.0mg

Directions
To one vial add 5ml of ethanol/sterile distilled water (1:1) and mix gently to dissolve the contents completely. Avoid frothing. Aseptically add the vial contents to 500ml of sterilised Listeria Selective Agar Base (Oxford Formulation) CM856, cooled to 50°C. Mix well and pour into sterile petri dishes.

MSRV SELECTIVE SUPPLEMENT

Code: SR161

A selective supplement for the detection of motile Salmonella spp when used with MSRV Medium Base CM910. See Section 2.

Vial contents (each vial is sufficient to supplement 500ml of medium)

Novobiocin	10mg

Directions
To one vial add 2ml of sterile distilled water and mix gently to dissolve. Avoid frothing. Aseptically add the vial contents to 500ml of MSRV Medium Base CM910 cooled to 50°C.

MYCOPLASMA SUPPLEMENT – G

Code: SR59

A selective supplement for the isolation of mycoplasmas when used with Mycoplasma Agar or Broth Base CM401/CM403. See Section 2.

Vial contents (each vial is sufficient for 80ml of medium)

Horse serum	20ml
Yeast extract (25% w/v)	10ml
Thallous acetate	25mg
Penicillin	20,000 IU

Directions
The sterile supplement is prepared by aseptically adding 20ml of sterile distilled water to the vial and mixing gently. Aseptically add the contents of the vial to 80ml of sterilised Mycoplasma Agar or Broth Base CM401/CM403, previously cooled to 50°C.

MYCOPLASMA SUPPLEMENT – P

Code: SR60

A selective supplement for the isolation of Mycoplasma pneumoniae when used with Mycoplasma Agar Base CM401. See Section 2.

Vial contents (per vial)

Horse serum	6ml
Yeast extract (25% w/v)	3ml
Thallous acetate	0.008g
Glucose	0.3g
Phenol red	0.0012g
Methylene blue	0.0003g
Penicillin	12,000 IU
Mycoplasma Broth Base CM403	0.146g

Directions
Prepare the sterile supplement by aseptically adding 20ml sterile distilled water to the vial and mix gently. Add 1ml of Mycoplasma Base CM401 without supplements to each of ten small bottles. Sterilise by autoclaving at 121°C for 15 minutes. Allow to set. Aseptically add 2ml of the reconstituted supplement P to each bottle containing agar.

OXYTETRACYCLINE GYE SUPPLEMENT

Code: SR73

A selective supplement for the isolation of yeasts and moulds when used with Oxytetracycline Glucose Yeast Extract Agar Base CM545. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Oxytetracycline (in a buffered base)	0.05g

NB: When re-constituted the resultant solution is photo-sensitive. It is recommended the solution is added immediately to the prepared agar base. Failure to do so may result in the solution becoming cloudy.

Directions
To one vial aseptically add 10ml of sterile distilled water and dissolve the powder completely. Add the contents to 500ml of Oxytetracycline-Glucose-Yeast Extract Agar Base CM545, cooled to 50°C. Mix thoroughly and pour into sterile petri dishes.

PALCAM SELECTIVE SUPPLEMENT

Code: SR150

A selective supplement for the isolation of Listeria monocytogenes when used with PALCAM Agar Base CM877. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Polymixin B	5mg
Acriflavine hydrochloride	2.5mg
Ceftazidime	10mg

Directions
To one vial add 2ml of sterile distilled water and mix gently to dissolve. Avoid frothing. Aseptically add the vial contents to 500ml of PALCAM Agar Base CM877 cooled to 50°C. Mix well and pour into sterile petri dishes.

PERFRINGENS (OPSP) SELECTIVE SUPPLEMENT A

Code: SR76

A selective supplement for the isolation of C. perfringens when used with SR77 and Perfringens Agar Base (OPSP) CM543. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Sodium sulphadiazine	50mg

Directions
To one vial add 2ml of sterile distilled water and dissolve the powder completely. Add the contents aseptically to 500ml of Perfringens Agar Base (OPSP) CM543 at 50°C. Mix well before pouring into sterile petri dishes.

PERFRINGENS (OPSP) SELECTIVE SUPPLEMENT B

Code: SR77

A selective supplement for the isolation of C. perfringens when used with SR76 and Perfringens Agar Base (OPSP) CM543. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Oleandomycin phosphate	0.25mg
Polymyxin B	5,000 IU

Directions
To one vial add 2ml of sterile distilled water and dissolve the powder completely. Add the contents aseptically to 500ml of Perfringens Agar Base (OPSP) CM543 at 50°C. Mix well before pouring into sterile petri dishes.

PERFRINGENS (SFP) SELECTIVE SUPPLEMENT

Code: SR93

A selective supplement for the isolation of C. perfringens when used with Perfringens Agar Base (TSC/SFP) CM587. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Kanamycin sulphate	6.0mg
Polymyxin B	15,000 IU

Directions
To one vial add 2ml of sterile distilled water and dissolve the powder completely. Add the contents aseptically to 500ml of sterile molten Perfringens Agar Base (TSC/SFP) CM587 cooled to 50°C, together with 25ml of Egg Yolk Emulsion SR47. Mix well before pouring into sterile petri dishes.

PERFRINGENS (TSC) SELECTIVE SUPPLEMENT

Code: SR88

A selective supplement for the isolation of C. perfringens when used with Perfringens Agar Base (TSC/SFP) CM587. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

D-cycloserine	200mg

Directions
To one vial add 2ml of sterile distilled water and dissolve the contents completely. Add the contents aseptically to 500ml Perfringens Agar Base (TSC/SFP) CM587 at 50°C. Mix well before use.

POST PASTEURISATION CONTAMINATION TEST SUPPLEMENT (PPCT)

Code: SR159

A selective supplement for use in the dairy industry.

Vial contents (each vial is sufficient for 200ml of medium)

Penicillin	4,000 IU
Nisin	8,000 units
Crystal violet	0.4mg

Directions
To one vial aseptically add 2ml of sterile distilled water and mix gently to dissolve completely. Add the contents to 200ml of sterile Milk Agar (CM21). Cool to 50°C. Mix gently and pour into sterile petri dishes.

STAPH/STREP SELECTIVE SUPPLEMENT

Code: SR70

A selective supplement for the isolation of staphylococci and streptococci when used with Columbia Agar Base CM331 or Blood Agar Base No.2 CM271. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Nalidixic acid	7.5mg
Colistin sulphate	5.0mg

This supplement is used with Columbia Agar Base and 5% defibrinated sheep blood to prepare Columbia CNA Agar.

Directions
To one vial add 5ml of 95% ethanol and mix gently to dissolve the powder completely. Add the contents aseptically to 500ml of Columbia Agar Base CM331 or Blood Agar Base No.2 CM271 cooled to 50°C. Mix well before pouring into sterile petri dishes.

STREPTOCOCCUS SELECTIVE SUPPLEMENT (COA)

Code: SR126

A selective supplement for the isolation of Streptococcus species when used with Columbia Agar Base CM331. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Colistin sulphate	5mg
Oxolinic acid	2.5mg

Directions
To one vial aseptically add 2ml of sterile distilled water. Aseptically add the contents of the vial to 500ml of sterile Columbia Blood Agar Base CM331 cooled to 50°C. Mix gently and pour into sterile petri dishes.

SULPHAMANDELATE SUPPLEMENT

Code: SR87

A selective supplement for the isolation of salmonellae when used with Brilliant Green Agar (Modified) CM329. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Sodium sulphacetamide	500mg
Sodium mandelate	125mg

Directions
To one vial aseptically add 5ml of sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Add the solution to 500ml of sterile Oxoid Brilliant Green Agar (Modified) CM329 cooled to 50–55°C. Mix gently and pour into sterile petri dishes.

TINSDALE SUPPLEMENT

Code: SR65

A selective supplement for the isolation and presumptive identification of C. diphtheriae when used with Tinsdale Agar Base CM487. See Section 2.

Vial contents (each vial is sufficient for 200ml of medium)

Serum	equiv. 20ml
Potassium tellurite	0.06g
Sodium thiosulphate	0.085g

Directions
To one vial aseptically add 15ml sterile distilled water and mix gently. Add this to 200ml of Tinsdale Agar Base CM487 previously cooled to 50°C.

THIS SUPPLEMENT IS HEAT SENSITIVE AND THE TEMPERATURE OF THE MOLTEN AGAR MUST NOT EXCEED 55°C AT THE TIME THE SUPPLEMENT IS ADDED.

VCAT SELECTIVE SUPPLEMENT

Code: SR104

A selective supplement for the isolation of pathogenic neisseria when used with GC Agar Base CM367. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Vancomycin	1.0mg
Colistin sulphate	3.75mg
Amphotericin B	0.5mg
Trimethoprim	1.5mg

Directions
To one vial aseptically add 10ml of sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Add the contents to 490ml of sterile GC Agar Base CM367, cooled to 50–55°C, containing 50ml Defibrinated Horse Blood SR50 lysed with 0.5% (v/v) saponin, and the rehydrated contents of one vial of Yeast Autolysate Supplement SR105.

VCN SELECTIVE SUPPLEMENT

Code: SR101

A selective supplement for the isolation of pathogenic neisseria when used with GC Agar Base CM367. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Vancomycin	1.5mg
Colistin sulphate	3.75mg
Nystatin	6,250 IU

Directions
To one vial aseptically add 2ml of sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Add the contents to 500ml of sterile Thayer Martin Medium, cooled to 50–55°C, prepared from GC Agar Base CM367, 1% (w/v) Soluble Haemoglobin Powder L53 plus the rehydrated contents of one vial of Vitox SR90.

VCNT SELECTIVE SUPPLEMENT

Code: SR91

A selective supplement for the isolation of pathogenic neisseria when used with GC Agar Base CM367. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Vancomycin	1.5mg
Colistin sulphate	3.75mg
Nystatin	6,250 IU
Trimethoprim	2.5mg

Directions
To one vial aseptically add 2ml of sterile distilled water and mix gently to dissolve the powder completely. Add the contents to 500ml of sterile Thayer Martin Medium, cooled to 50–55°C, prepared from GC Agar Base CM367, 1% (w/v) Soluble Haemoglobin Powder L53 and the rehydrated contents of one vial of Vitox SR90.

YEAST AUTOLYSATE GROWTH SUPPLEMENT

Code: SR105

A growth supplement for the enrichment of pathogenic neisseria when used with GC Agar CM367. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Yeast autolysate fractions	5.0g
Glucose	0.5g
Sodium bicarbonate	0.075g

Directions
Aseptically add 15ml of sterile distilled water to a vial and shake vigorously until the contents are completely dissolved.

YERSINIA SELECTIVE SUPPLEMENT

Code: SR109

A selective supplement for the isolation of Y. enterocolitica when used with Yersinia Selective Agar Base CM653. See Section 2.

Vial contents (each vial is sufficient for 500ml of medium)

Cefsulodin	7.5mg
Irgasan	2.0mg
Novobiocin	1.25mg

Directions
To one vial aseptically add 1ml of ethanol to 2ml of sterile distilled water. Mix gently to dissolve the contents completely. Aseptically add the contents to 500ml of sterile Oxoid Yersinia Selective Agar Base CM653 cooled to 50°C. Mix gently and pour into sterile petri dishes.

RPF SUPPLEMENT

Code: SR122

A selective and diagnostic supplement for the isolation, enumeration and confirmation of Staphylococcus aureus from food and other specimens. See Section 2.

Formula (each vial is sufficient for 100ml of medium)

Fibrinogen	0.375g
Rabbit plasma	2.5ml
Trypsin inhibitor	2.5mg
Potassium tellurite	2.5mg

Directions
To one vial aseptically add 10ml of distilled water. Turn vial end-over-end to dissolve. Avoid frothing the solution. Dissolution is not obtained immediately. Leave for one to two hours to dissolve completely. Aseptically add the vial contents to 90ml of sterile Baird-Parker Base (Oxoid CM275) cooled to 48°C. Mix well and use immediately.

Description
Rabbit Plasma Fibrinogen Agar (RPF Agar) is based on the formulation described by Beckers et al[1]. This medium is a modification of Baird-Parker Medium and is recommended for the selective isolation, enumeration and confirmation of *Staphylococcus aureus* from food and other specimens[2].

The RPF Agar formulation retains the Baird-Parker Agar Base which has been specifically formulated to resuscitate injured cells[3]. This medium differs from Baird-Parker Medium in that the egg yolk emulsion has been replaced by fibrinogen, rabbit plasma and trypsin inhibitor. The fibrinogen was added to enhance the coagulase reaction in the RPF Agar[4]. Rabbit plasma was selected and it was found to be more specific for the coagulase activity when compared to other sources of plasma[1]. Trypsin inhibitor was added to prevent fibrinolysis.

The RPF Agar supplement has been modified in one respect from the original formulation in that the potassium tellurite content has been reduced four-fold, i.e. from 0.01% to 0.0025% w/v. This reduction was necessary as it was discovered in the Oxoid laboratory that some strains of *Staphylococcus aureus*

were sensitive to potassium tellurite when used at 0.01% w/v in RPF Agar[5]. This modification of RPF Agar was found to give comparable growth and selectivity to that achieved on Baird-Parker Medium. The improved productivity of RPF Agar has also been confirmed by other laboratories[6,7]. The reduction in potassium tellurite concentration in RPF Agar results in *Staphylococcus aureus* strains forming white or grey or black colonies, which are surrounded by an opaque halo of precipitation, i.e. the coagulase reaction.

Technique

Surface Inoculation Method

1 Prepare the RPF Agar plates as directed.
2 Process the food sample in a stomacher or Waring blender using the recommended sample size and diluent.
3 Separate plates are inoculated with 0.1ml of the prepared samples and the subsequent decimal dilutions of them.
4 Incubate at 35°C and examine after 24 and 48 hours incubation.
5 Count all the colonies that have an opaque halo of precipitation around them. Do not limit the count to black colonies.
6 Report as number of coagulase positive staphylococcus isolated per gram of food.

Pour Plate Method

1 Prepare the RPF Agar as directed and hold at 48°C.
2 Process the food sample in a stomacher or Waring blender using the recommended sample size and diluent.
3 Add 1ml of the prepared sample (initial suspension and subsequent decimal dilution) into each sterile petri dish.
4 Add aseptically 20ml of sterile RPF Agar and prepare pour plates.
5 Incubate at 35°C and examine after 24 to 48 hours.
6 Count all the colonies that have an opaque halo of precipitation around them.
7 Report as number of coagulase positive staphylococcus isolated per gram of food.

Precautions

Colonies of some contaminating organisms growing in close proximity to the coagulase positive colonies may partially digest the coagulase halo reaction.

References

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4 Hauschild A. H. W., Park C. E. and Hilsheimer R. (1979) *Can. J. Microbiol. 25. 1052–1057.*
5 Sawhney D. (1986) *J. Appl. Bact. 61. 149–155.*
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VITOX

Code: SR90

Contents (per vial)

Vitamin B_{12}	0.1mg
L-glutamine	100.0mg
Adenine	10.0mg
Guanine	0.3mg
p-Aminobenzoic acid	0.13mg
L-cystine	11.0mg
NAD (Coenzyme 1)	2.5mg
Cocarboxylase	1.0mg
Ferric nitrate	0.2mg
Thiamine	0.03mg
Cysteine hydrochloride	259.0mg

Description

Vitox is a sterile lyophilised concentrate of essential growth factors. Many workers prefer such a chemically defined growth supplement to yeast extract for the supplementation of Thayer Martin Medium.

VITOX HYDRATION FLUID

Code: SR90B

Contents (per vial)

Glucose	1.0g
Distilled water	10.0ml

Description

Vitox may be used satisfactorily at a final concentration of 1% v/v in culture media (1 vial to 1,000ml of medium).

However, it was found at Oxoid Laboratories that increasing the concentration to 2% v/v (1 vial to 500ml of medium) in Thayer Martin Medium resulted in faster growth of *Neisseria gonorrhoeae*. Use at 2% v/v is therefore recommended.

In an evaluation of media for primary isolation of *Helicobacter pylori* from gastric biopsy specimens Vitox was found to increase the isolation rate[1].

1 Piccolomini R., Di Bonaventura G., Festi D. et al. (1997) *J. Clin. Microbiol. 35. 1541–1544.*

STERILE REAGENTS

Sterile reagent products are offered in ready-to-use form as enrichment solutions, essential growth factors, enzyme substrates or biochemical indicators.

Each product has been processed with an appropriate sterilisation procedure, e.g. aseptic preparation, filtration or irradiation, which will not affect the performance of the product. All sterile reagents should be stored at 2–8°C away from light.

EGG YOLK EMULSION

Code: SR47

Description

A sterile stabilised emulsion of egg yolk for use in culture media. It may be added directly to nutrient media for the identification of *Clostridium*, *Bacillus* and *Staphylococcus* species by their lipase activity.

Technique
Examination of Bacteria for Lecithinase
For demonstration of lecithinase activity (especially in the investigation of 'bitty cream' conditions) add 0.5 or 1.0ml of the emulsion to 10ml of sterile Blood Agar Base CM55 or Nutrient Broth No.2 CM67 – in both cases to clear the medium, raise the final salt concentration by the addition of 1% of sodium chloride. After incubation for up to 5 days at 35°C, lecithinase-producers render the broth opalescent, whilst, on the solid medium, their colonies are surrounded by zones of opacity.

EGG YOLK TELLURITE EMULSION

Code: SR54

Description
A sterile emulsion of egg yolk containing potassium tellurite for use in Baird-Parker Medium CM275. Baird-Parker Medium is widely used in the food industry for the detection of pathogenic staphylococci. Baird-Parker plates incorporating Egg Yolk Tellurite Emulsion should be protected from moisture loss by enclosure in plastic or other vapour proof packaging.

Directions
Add 50ml to 1 litre of Baird-Parker Medium CM275. (50ml Egg Yolk Tellurite Emulsion contains the equivalent of 3ml of 3.5% potassium tellurite. This is the amount recommended for 1 litre of Baird-Parker Medium, i.e. concentration in SR54 is 0.21% w/v. Final concentration in Baird-Parker Medium is 0.01% w/v.)

FILDES PEPTIC DIGEST OF BLOOD

Code: SR46

Description
Fildes Extract is prepared by the action of the enzyme pepsin on defibrinated horse blood at optimum temperature and pH value, as described by Fildes[1]. It is a rich source of growth factors, including haematin and coenzyme, derived from the blood cells from which it is prepared. As some of the growth factors are thermolabile it should not be heated above 55°C.

The extract is supplied in sterile screw-capped bottles. Add to the appropriate medium only after the medium has been sterilised.

Technique
Fildes Extract is recommended for the preparation of many culture media among which are the following examples:

Nagler Medium
Melt 100ml sterile nutrient agar (Blood Agar Base CM55 is recommended) and cool to 50°C. Add 5ml of Fildes Extract and 20ml sterile serum or plasma. Pour plates and dry. Concentrated Egg Yolk Emulsion SR47 may be used in 5% concentration.

Fildes Broth (Fildes Peptic Blood Broth)
Add 5ml of Fildes Extract to 100ml of sterile Nutrient Broth No.2 CM67 or other liquid nutrient media.

Fildes Agar (Fildes Peptic Blood Agar)
Melt 100ml of nutrient agar or Blood Agar Base CM55, cool to 50°C and add 5ml of Fildes Extract.

Fildes Broth and Agar, which are transparent and have the colour of nutrient broth or agar, give copious growths of *Haemophilus influenzae* and are admirably suited for the primary isolation of this organism.

Lactose Egg-Yolk Milk Agar[1,2] – a medium for the identification of anaerobes which, in addition to serving as a half antitoxin-Nagler plate, also demonstrates lactose fermentation and proteolysis. Egg Yolk Emulsion SR47 is recommended for use in the preparation of the medium.

References
1 Willis A. T. and Hobbs G. (1959) *J. Path. Bact. 77. 511–521.*
2 Willis A. T. (1977) *Anaerobic Bacteriology 3rd Edn. Butterworths, London.*

TOMATO JUICE

Code: SR32

Description
This product is the juice of ripe tomatoes, clarified and sterilised by filtration. It has a pH of 4.1 (approx) and 100ml of SR32 is equivalent to 227 grams of tomato. Tomato juice can be added to nutrient media as a specific growth stimulant for lactic acid bacteria.

STERILE SOLUTIONS OF CHEMICAL PRODUCTS FOR CULTURE MEDIA

LACTIC ACID 10%

Code: SR21

POTASSIUM LACTATE 50%

Code: SR37

POTASSIUM TELLURITE 3.5%

Code: SR30

UREA 40%

Code: SR20

FREEZE-DRIED STERILE REAGENTS FOR BACTERIOLOGICAL PURPOSES

BROAD SPECTRUM BETA-LACTAMASE MIXTURE

Code: SR113

Broad Spectrum Beta-Lactamase Mixture SR113 is a mixture of beta-lactamase (E.C.3.5.2.6.) from *Bacillus cereus*[1] 569/H9.

The enzymes are presented as a sterile freeze-dried powder containing buffer and zinc salts. Each vial contains minima of 500 units beta-lactamase I and 50

units of beta-lactamase II. 1 unit of enzyme activity will hydrolyse 1 μmol of substrate per minute at pH 7.0 and at 25°C; beta-lactamase I is assayed using benzyl penicillin in the presence of EDTA, and beta-lactamase II using cephalosporin C in the presence of Zn^{2+}.

Definition of Units of Enzyme Activity
The scientific literature describes a number of methods which are used to measure and define a unit of penicillinase of beta-lactamase activity[2]. Note that 1 IU of activity = 600 Levy units of activity.

Application
There are four major uses of this preparation of enzymes.

1 Inactivation of beta-lactam antibiotics in blood or other tissue samples prior to routine microbiological examination[2,3,4].
2 Inactivation of beta-lactam antibiotics in blood and other tissue samples prior to the microbiological estimate of aminoglycosides or other non-lactam antibiotics[4,5].
3 The inactivation of beta-lactam antibiotic preparations to enable sterility testing to be carried out before the administration of such preparations to patients undergoing therapy with immuno-suppressants, or who have a naturally low level of immunity[6].
4 Assessment of the susceptibility of new beta-lactam antibiotics to inactivation by lactamase.

Methods
1 ***Blood Culture Procedures***

Inject 5ml of sterile distilled water into a vial of enzyme mixture and mix gently. Add 1ml of this solution aseptically to the blood culture bottle, preferably before or immediately after inoculation with the blood sample (5–10ml).

2 ***Microbiological Assay of Non-Lactam Antibiotics***

1ml of the beta-lactamase enzyme solution should be added aseptically to 1ml of blood sample or serum. This should be incubated at 30°C for a period of time depending on the beta-lactam antibiotic present. A minimum time would be 5 minutes and a maximum 60 minutes. After incubation, the blood or serum samples should be applied to wells in previously seeded antibiotic assay plates in the normal manner.

Stability of Reagents
Solutions of the enzyme will remain active for several days when stored at 4°C or several weeks when stored at minus 20°C.

Repeated freezing and thawing should be avoided. However, it is not advisable to store the solution for long periods because of the possibility of contamination.

References

1 Davis R. B., Abraham E. P. and Melling J. (1974) *Biochem. J. 143. 115–127.*
2 Waterworth P. M. (1973) *J. Clin. Path. 26. 596–598.*
3 Selwyn S. (1977) *J. Antimicrob. Chemother. 3. 161–168.*
4 Newson S. W. B. and Walshingham B. M. (1973) *J. Med. Microbiol. 6. 59–66.*
5 Sabath L. D., Casey J. I., Ruch P. A., Stumpf L. L. and Finland M. (1971) *J. Lab. Clin. Med. 78. 457–463.*
6 Code of Federal Regulations, Title 21, Part 436, Sec.436.20 U.S. Govt. Printing Office, Washington, D.C.

NITROCEFIN (GLAXO RESEARCH 87/312)

Code: SR112, SR112A

For the rapid chromogenic detection of beta-lactamase activity.

Reagents
SR112
Vial of lyophilised Nitrocefin, containing 1mg Nitrocefin.

SR112A
Rehydration fluid. The vial contains 1.9ml of phosphate buffer (0.1M, pH 7.0) and 0.1ml of dimethylsulphoxide.

Directions
Reconstitute the contents of one vial of lyophilised Nitrocefin SR112 by adding the entire contents (2ml) of one vial of rehydration fluid SR112A. This yields a working Nitrocefin solution of 500μg/ml, (approx 10^{-3} M) suitable for most applications.

Precautions
Nitrocefin, particularly in solution, is very light sensitive. The solution may be stored at –20°C for up to two weeks. INGESTION OR INHALATION, OR CONTACT WITH THE SKIN AND EYES SHOULD BE AVOIDED.

General Introduction and Intended Uses
Nitrocefin is the chromogenic cephalosporin developed by Glaxo Research Limited. (Coded 87/312; 3-(2,4 dinitrostyrl) – (6R,7R–7–(2-thienylacetamido)–ceph–3–em–4–carboxylic acid, E-isomer)[1].

This compound exhibits a rapid distinctive colour change from yellow (max at pH 7.0 = 390nm) to red (max at pH 7.0 = 486nm) as the amide bond in the beta-lactam ring is hydrolysed by a beta-lactamase (E.C 3.5.2.6); it is sensitive to hydrolysis by all known lactamases produced by Gram-positive and Gram-negative bacteria. This characteristic reaction forms the basis of a number of methods suitable for diagnostic use.

Apart from its use in giving rapid indication of beta-lactamase potential, the reagent has been found extremely useful for the detection of beta-lactamase patterns from bacterial cell extracts on iso-electric focusing[2,3,4] and has been used in inhibition studies in development work on beta-lactamase resistant antibiotics[5].

Description of Use

Demonstration of beta-lactamase activity in bacterial cells.
Nitrocefin degradation should be used to give a rapid indication of beta-lactam inactivating systems and the result so obtained will, in most cases, predict the outcome of susceptibility tests with beta-lactam anti-microbials. However, it should not entirely replace conventional susceptibility testing as other factors

also influence the results of such tests, and on occasion intrinsic resistance to beta-lactam antimicrobials has not been correlated with production of beta-lactamase[6].

Nitrocefin degradation has been found to be highly efficient in detecting beta-lactamase producing isolates of *Neisseria gonorrhoeae*[7,8], *Haemophilus influenzae*[7,9,10,11] and *staphylococci*[10,11].

Excellent results have also been obtained with certain anaerobic bacteria, notably with *Bacteroides* species[13,14,15]. It should be emphasised that the efficacy of the Nitrocefin tests in predicting the beta-lactam susceptibilities of other micro-organisms is at present unproven.

Another chromogenic cephalosporin, PADAC (Hoechst-Roussel) was not as effective as Nitrocefin in detecting staphylococcal beta-lactamase[12].

Technique
Rehydrate the Nitrocefin as directed, and use this solution in the following ways:

1 ***Direct Plate Method***[1]

Add one drop of the Nitrocefin solution on to the surface of the colony. If the isolate is a high beta-lactamase producer then the colony and the surrounding area will quickly turn red.

To detect a weak beta-lactamase producer the plate should then be incubated for 30 minutes before being reported as negative.

2 ***Slide Method***[1]

Add one drop of the Nitrocefin solution on to a clean glass slide. Using a sterile loop, pick one colony from the plate and emulsify into the Nitrocefin drop. Report as positive if the colour changes from yellow to red within 30 minutes (protect the slide from desiccation during the waiting period).

3 ***Broth Method***[1]

Add four drops of Nitrocefin solution to 1ml of the grown culture. Report as positive if the colour changes to red within 30 minutes.

4 ***Broken Cell Method***[1]

Sonicate 1ml of the culture in order to break open the cells. Add 4 drops of Nitrocefin solution. Report as positive if the colour changes to red within 30 minutes.

5 ***Paper Disc Spot Test***[10]

A Whatman No.1 filter paper disc (diameter 7cm) is placed in a petri dish and impregnated with Nitrocefin solution (0–5ml). This impregnated paper is generally usable for one day, but should be kept away from light to avoid spontaneous degradation. An isolated colony is applied to the impregnated paper with a loop; a pink to red reaction developing within 15 minutes indicates beta-lactamase presence.

Detection of beta-lactamase activity on gels
Methods for preparing extracts containing the beta-lactamase activities from bacterial cells and the technique for analytical iso-electric focusing have been described by Matthew et al.[2] The developed gels are stained by applying Whatman No.54 paper impregnated with the Nitrocefin solution[2]. Focused bands in the gel with beta-lactamase activity appear pink on a yellow background.

Determination of beta-lactamase activity by spectrophotometric assay
The working solution of Nitrocefin (500mg/ml) is diluted tenfold in buffer (0.1M phosphate; 1mM EDTA, pH 7.0). Spectrophotometric assays for beta-lactamase are carried out measuring changes in wavelength at 486nm. The molar extinction coefficient of Nitrocefin at this wavelength is 20,500.

Test samples of the finished product for performance with control cultures.

References

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12 Anhalt J. P. and Nelson R. (1982) *Antimicrob. Ag. & Chemother. 21. 993–994.*
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14 Timewell R., Taylor E. and Phillips I. (1981) *J. Antimicrob. Chemother. 7. 137–146.*
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PENASE

Code: SR129

569/H9 Lactamase active against a range of penicillins.

Materials Supplied
Penase SR129 is a *Bacillus cereus* 569/H9 lactamase (E.C.5.2.6) presented as a sterile freeze-dried powder containing buffer salts. Each vial contains 3,300 IU of activity (1 unit of enzyme activity will hydrolyse 1.0 μmol of benzylpenicillin to benzylpenicilloic acid per minute pH 7.0 and at 25°C). The preparation will successfully inactivate a range of penicillins[1].

Definition of Units of Enzyme Activity
The scientific literature describes a number of methods which are used to measure and define a unit of penicillinase activity[2]. Note that 1 IU of activity = 600 Levy units of activity.

Application
The major use of this enzyme preparation is for the inactivation of susceptible beta-lactam antibiotic

preparations to enable sterility testing to be carried out[3].

The preparation may also be used for inactivation of susceptible antibiotics in blood or other tissue samples prior to routine microbiological examination[2,4,5,] and inactivation of susceptible antibiotics in blood and other tissue samples prior to the microbiological estimation of aminoglycosides or other non beta-lactam antibiotics[6].

Methods

1 ***Sterility Testing of Penicillin Products***[3]
The product is rehydrated by adding 5ml of sterile distilled water to a vial of enzyme with gentle mixing. The resulting solution will contain 660 IU of activity/ml.

The sterility of penicillin products with respect to bacterial contamination is determined by adding 300mg or less of the test sample to sterile Fluid Thioglycollate Medium CM173 or other prescribed media to which a suitable amount of Penase solution has been added aseptically when the temperature has fallen below 50°C. Tubes (35mm x 200mm) containing 90–100ml medium are incubated for 7 days at 30°C.

The sterility of penicillin products with respect to yeast and moulds is accomplished by adding the test sample to Sabouraud Liquid Medium CM147 and incubating for 7 days at 20–25°C.

2 ***Blood Culture Procedures***
0.1ml of the reconstituted solution should be added aseptically to blood culture broths, preferably before but otherwise immediately after inoculation with the blood sample.

Stability of Reagents

Solutions of the enzyme will remain active for several days when stored at 4°C or several weeks when stored at minus 20°C. Repeated freezing and thawing should be avoided. However, it is not advisable to store the solution for long periods because of the possibility of contamination.

References

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2 Newson S. W. B. and Walshingham B. M. (1973) *J. Med. Microbiol. 6. 59–66.*
3 Code of Federal Regulations, Title 21, Part 436, Sec. 436.20. U.S. Govt. Printing Office, Washington, DC.
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6 Sabath L. D., Casey J. I., Ruch P. A., Stumpf L. L. and Finland M. (1971) *J. Lab. Clin. Med. 78. 457–463.*

SPUTASOL

Code: SR89

Formula (per vial)

Dithiothreitol	0.1g
Sodium chloride	0.78g
Potassium chloride	0.02g
Disodium hydrogen phosphate	0.112g
Potassium dihydrogen phosphate	0.02g
pH 7.4 ± 0.2	

Directions

To one vial aseptically add 5ml of sterile distilled water and mix gently to dissolve the contents completely. Aseptically add the contents to 95ml of sterile distilled water. Use immediately or store at 4°C for 48 hours only.

Description

Sputum generally consists of inflammatory exudate from the lower respiratory tract mixed with saliva.

Mulder[1] recognised the problem of interpreting the significance of growth from sputum and suggested. rinsing it in saline before culture to remove the saliva. May[2] showed that bacteria are often unevenly distributed in the sputum of patients suffering from chronic bronchitis and that single cultures may fail to reveal all the bacterial species present.

The introduction by Rawlins[3] of a method for the homogenisation of sputum before culture overcame the variations present in any method that is based on the examination of small proportions of heterogeneous material. It enables the bacteria in the sputum to be distributed evenly throughout the specimen after digestion. Dixon and Muller[4] in an attempt to distinguish between contaminants and bronchial pathogens, suggested a semi-quantitative analysis by diluting the digested sputum down to 10^{-4}.

Dithiothreitol, Cleland's Reagent[5], has been evaluated as a sputum liquefying agent[6]. It was found the most effective of a group of agents tested containing a sulphydryl group.

A 0.1m solution of dithiothreitol was found to achieve a significantly greater decrease in sputum viscosity than 1.2M N-acetyl cysteine for use prior to sputum culture.

The use of dithiothreitol instead of N-acetyl cysteine to digest sputum before decontamination has been shown[7] to yield a higher number of acid-fast bacilli when smears are stained by the Ziehl-Neelsen method. After culture and incubation for three weeks it was reported that in general the number and size of colonies isolated using dithiothreitol as a liquefying agent was greater than that using N-acetyl cysteine.

Technique

The procedure for the routine liquefaction of sputum is as follows:

1 The sputum is expectorated into a sterile Universal container or other wide mouthed screw-capped bottle.
2 Add approximately 5 times the volume of 0.85% saline and agitate to free the sputum from adherent saliva. Remove the saline with a sterile Pasteur pipette.
3 To the washed sputum, add an equal volume of Sputasol solution.
4 Shake the mixture well, place in a 37°C water bath and incubate, with periodic shaking, until liquifaction is complete.
5 Inoculate on to a suitable culture medium. For the total cell count, place a drop of the liquefied sputum in a haemocytometer for enumeration. For

a differential cell count, fix a dried smear in methyl alcohol and stain with haematoxylin and eosin or with Lieshmann stain.

Reconstituted solutions of Sputasol, if kept sterile, are stable for at least 48 hours stored at 2–8°C.

An investigation into the survival of respiratory pathogens in specimens that had been stored for 48 hours at 4°C following homogenisation using Sputasol, showed that the organisms remained viable and, when necessary, treated specimens could be succesfully re-cultured[8].

References

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TTC SOLUTION (0.05%)

Code: SR148

TTC Solution is supplied as 5ml of filter-sterilised aqueous solution of tri-phenyltetrazolium chloride (TTC). It is used to supplement Tergitol-7 Agar CM793 for the enumeration of coliforms in food and water supplies.

READY PREPARED MEDIA AND DIP SLIDES

Coded PM, R and DS

These products are supplied in a form which is ready for inoculation and incubation.

Storage conditions and Shelf life
PM and R products should be stored at 2–8°C. DS products can be stored at low room temperature. All products should be used before their expiry date shown on the label.

Quality Control
These products have been prepared and tested to high standards. Microbiological control tests, using appropriate organisms, are made and the product is released only if it satisfies these tests. If the medium is stored as directed and used within the expiry date, then further tests are not required. However, if it is to be used for purposes other than those described below, then it would be wise to make suitable control tests to ensure growth of the expected organism.

Precautions
As with all culture media, these products should be used by trained staff only, in suitable laboratories and disposed of safely by autoclaving at 121°C for at least 20 minutes.

Mycobacterium tuberculosis (incl. *M. bovis*) is classified as a hazardous organism, specimens and cultures must be processed in contained laboratories, using approved protective cabinets. Staff should be specially trained, tested for adequate immunity and medically examined at appropriate intervals.

ACTIDIONE® AGAR

Code: PM118

Formula	gm/litre
Yeast extract	4.0
Tryptone	5.0
Glucose	50.0
Potassium dihydrogen phosphate	0.55
Potassium chloride	0.425
Calcium chloride	0.125
Magnesium sulphate	0.125
Ferric chloride	0.0025
Manganese sulphate	0.0025
Bromocresol green	0.022
Cycloheximide	0.01
Agar	15.0
Distilled water to 1 litre	
pH 5.5 ± 0.2	

Description
Actidione® (cycloheximide) at a concentration of 0.001% w/v permits the growth of bacteria but inhibits the growth of most yeasts and moulds except dermatophytes. Media containing this antibiotic are invaluable for the enumeration and detection of bacteria in specimens containing large numbers of yeasts and moulds. For example, the medium is used for the estimation of bacterial contamination of pitching yeast. 'Actidione' Agar with added penicillin and streptomycin is also valuable as a selective medium for the isolation of dermatophytes.

The Oxoid medium, based on that of Green and Gray[1], may be used for microbiological investigations during brewing and baking. As an approximate guide, incubation may be at 25°C or 30°C for up to 14 days, according to the flora present. Green and Gray employed their medium at two different reactions, pH 5.5 and pH 6.5, the latter may be attained by adding approximately 16ml of sterile 1.5% sodium carbonate to each litre of molten medium.

Reference

1 Green S. R. and Gray P. P. (1950) *Wallerstein Lab. Communication 13. 357.*

SELENITE BROTH

Code: R39

Formula	gm/litre
Peptone	5.0
Lactose	4.0
Sodium biselenite	4.0
Sodium phosphate	10.0
Distilled water to 1 litre.	
pH 7.1 ± 0.2	

Description
Selenite Broth in ready-to-use form is prepared from Selenite Broth Base CM395 with Sodium Biselenite L121.

It is a modification of the original Leifson[1] selenite broth F, used as an enrichment broth for the isolation

of salmonellae from samples of faeces, urine or other suspected material.

Incubation at 43°C will improve the selectivity of the medium when large numbers of contaminating bacteria are present[2].

References

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TRIBUTYRIN AGAR

Code: PM4

Formula	gm/litre
Peptone	5.0
Yeast extract	3.0
Tributyrin (glyceryl tributyrate)	10.0
Agar	15.0
Distilled water to 1 litre	
pH 7.5 ± 0.2	

Description

Tributyrin Agar which consists of a stable homogenate of nutrient agar and tributyrin (glyceryl tributyrate) is used for the detection and enumeration of lipolytic (fat-splitting) bacteria and moulds.

The Oxoid medium is also frequently used for the detection of the lipolytic activity of specific organisms such as staphyloccoci, clostridia, pseudomonads and flavobacteria[1,2,3,4,5,6].

Technique

For use, it is normally sufficient to prepare 1/10, 1/100, 1/1,000 and 1/10,000 homogenates of the melted butter in quarter strength Ringer Solution and, within 10 minutes, to transfer 1ml quantities of each dilution to separate sterile petri dishes. Add 10ml of the medium, melted and cooled to 45–48°C, mix, and incubate at 30°C for three days. After incubation, the medium appears opaque but lipolytic colonies are surrounded by a zone of clear medium.

References

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TRICHOMONAS MEDIUM NO.2

Code: R27

Formula	gm/litre
Liver digest	18.0
Glucose	20.0
Chloramphenicol	0.125
Tryptone soya broth CM129	30.0
Horse serum SR35	250ml
Calcium pantothenate 0.5% w/v	1ml
Distilled water	1,000ml
pH 6.2 ± 0.2	

Description

Trichomonas Medium No.2 is a modification of the media described by Bushby and Copp[1] and Squires and McFadzean[2] for the isolation of *Trichomonas vaginalis.*

Clinical trials have established that Trichomonas Medium No.2 is very successful for the culture of *Trichomonas vaginalis* from primary isolates. A combination of laboratory culture and slide examination provides the best chance of establishing the presence of this pathogen.

Trichomonas Medium No.2 contains both serum and antibiotic and is therefore ready for immediate inoculation. It will store at 4°C for several months without deterioration.

Technique

1 Inoculate the medium directly with vaginal swab, sub-preputial scrape, urethral scrape, prostatic fluid, or the deposit from lightly centrifuged urine[3].

2 Incubate at 34°C[4].

3 Examine microscopically every 24 hours.

4 Incubate microscopically negative cultures up to five days.

References

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METHODS AND MEDIUM FOR THE CULTURE OF TUBERCLE BACILLI

(F. Zaher and J. Marks[1])

MEDIA FOR THE ISOLATION OF TUBERCLE BACILLI

Method	Digestion Technique	Reaction of Inoculum	Zaher & Marks New Media[1] and Codes	New Oxoid Media
Simple	Mix specimen 1:1 with 4% NaOH for digestion. No centrifuging before inoculation.	Highly alkaline pH	Acid Egg Medium 1a	Modified Acid Egg Medium PM95
			Acid Egg Medium 1b	Modified Pyruvic Acid Egg Medium PM96
Standard Reference	Mix specimen 1:1 with 4% NaOH for digestion then further dilute total volume 1:4 in distilled water. Centrifuge before inoculation.	Moderately alkaline pH	Acid Egg Medium 2a	Standard Ref Acid Egg Medium PM99
			Acid Egg Medium 2b	Standard Ref Pyruvic Acid Egg Medium PM100
Petroff	Mix specimen 1:1 with 3% or 4% NaOH for digestion then centrifuge and neutralise before inoculation.	Neutral to slightly acid pH	Simplified L-J Medium Marks 2a (without HCl).	Simplified Lowenstein-Jensen PM97
			Simplified L-J Medium Marks 2b (without HCl).	Simplified, Pyruvate Lowenstein-Jensen PM98
Sulphuric Acid	Variable technique utilising H_2SO_4 concentrations of 2–5% for time periods 2–30 minutes dependent on specimen and degree of contamination. Used for specimens with small amounts of solids after centrifugation e.g. urines, CSF, pleural effusion.	Highly acid pH	Simplified L-J Medium Marks 2a (without HCl)	Simplified Lowenstein-Jensen PM97
			Simplified L-J Medium Marks 2b (without HCl)	Simplified, Pyruvate Lowenstein-Jensen PM98

LOWENSTEIN-JENSEN MEDIUM

Code: PM1

Formula	gm/litre
Potassium dihydrogen phosphate	2.4
Magnesium sulphate	0.24
Magnesium citrate	0.6
Asparagine	3.6
Glycerol	12.0ml
Potato starch	30.0
Fresh egg mixture	1,000ml
Malachite green	0.4
Distilled water to 1640ml	

Description
An inspissated egg medium for the cultivation and differentiation of *Mycobacterum* species, especially *M. tuberculosis* and *M. bovis*. Growth of *M. tuberculosis* is enhanced by the incorporation of glycerol, whilst the growth of *M. bovis* and certain other mycobacteria is not enhanced and may even be inhibited. The Oxoid medium corresponds to the modification of the Lowenstein[1] medium developed by Jensen[2].

Technique
The specimen is inoculated directly on to the surface of the medium; material which is contaminated with other micro-organisms, or which contains few mycobacteria, may be subjected to one of the numerous concentration methods before culturing.

It is preferable to inoculate duplicate slopes of medium with each specimen. Incubate at 35°C and examine after 10 to 14 days, and then every week until the culture is discarded after a total of 8 weeks incubation. *Mycobacterium tuberculosis* grows well to produce large dry, heaped-up yellowish colonies, whilst *M. bovis* fails to grow or is only able to produce small, 'colourless', discrete colonies. For the cultivation of glycerol-sensitive mycobacteria, such as some strains of *M. bovis*, this medium is available as Lowenstein-Jensen Medium (without glycerol) PM2.

References

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ACID EGG MEDIUM

Code: PM1a

Formula	gm/litre
Potassium dihydrogen phosphate	12.3
Magnesium sulphate	0.3
Glycerol	12.0ml
Potato starch	30.0
Fresh egg mixture	1000ml
Malachite green	0.4
Penicillin G	100,000 IU
Distilled water to 1640ml	

Description
A solid medium for use in a simplified method of *Mycobacterium tuberculosis* culture, described by Marks[1]. The method entails the use of an acid egg medium which may be inoculated with an alkaline sputum homogenate which has been neither neutralised nor centrifuged. This saves time,

eliminates the need for an expensive centrifuge and reduces the risk of disseminating tubercle bacilli. The volume of medium (4.5ml) was designed to neutralise the alkali in 0.2ml of homogenate and this ratio should not be altered. Marks considered that his method was considerably more sensitive, and possessed a much lower contamination rate than those described by the International Union against Tuberculosis[2] and by Nassau[3] and summarises 'With the conditions recommended, this method is considered to be 99% as effective as a full-scale method with film-positive specimens and 98% with film-negative specimens'.

Technique (Marks[1])

1 Distribute 2ml amounts of sterile 4% NaOH into sterile 1oz. universal containers.

2 With a sterile plugged glass tube, pipette 2ml amounts of sputum into the NaOH, avoiding contact with the top half of the bottles. With scanty specimens, first add sterile distilled water to the NaOH equivalent to the volume deficiency.

3 Shake the bottles briefly in a rack, avoiding contamination of the caps, and place in a 35°C dry incubator, shake for 20 seconds after 7½, 15 and (if necessary) 22½, minutes. Inspect the mixtures after 15 and 22½, minutes, and each time immediately inoculate any which have become watery (ignore very small particles). Inoculate after 30 minutes' incubation, whatever the consistency. There is thus a minimum of 15 and a maximum of 30 minutes incubation during homogenisation.

4 On their withdrawal from the incubator, inoculate the alkaline homogenates directly into the Acid Egg Medium. For pipettes use sterile plugged glass tubes with an internal diameter of 4mm and marked corresponding to 0.2ml before sterilisation.

   Inoculate the Acid Egg Medium with 0.2ml of homogenate and tilt so that the inoculum runs over the entire surface of the slope.

5 Incubate at 35°C and examine every week until the culture is discarded after a total of 8 weeks' incubation.

References

1 Marks J. (1959) *Mon. Bull. Min. Hlth Pub. Hlth Lab. Serv. 18. 81–86.*

2 Report (1955) *Bull. Int. Union Tuberc. 25. 89.*

3 Nassau E. (1958) *Tubercle 39. 18–23.*

PYRUVIC ACID EGG MEDIUM

Code: PM2A

Formula	gm/litre
Fresh egg mixture	1000ml
Pyruvic acid	3.0 '
Potassium dihydrogen phosphate	11.4
Disodium hydrogen phosphate	6.0
Magnesium sulphate	0.3
Malachite green	0.125
Penicillin G	100,000 IU
Distilled water to 1640ml	

Description

Stonebrink[1,2], demonstrated the value of pyruvic acid in the cultivation of mycobacteria and presented evidence of enhanced growth of *Myco. bovis* and dysgonic strains of *Myco. tuberculosis.*

Marks[3] extended this work and confirmed the findings of Stonebrink, especially in the presence of reduced malachite green concentration. The Oxoid medium is made to Marks' formula.

Although Pyruvic Acid Egg Medium is superior to glycerol medium, Marks comments that the use of both media is to be recommended. This is because pyruvic acid may discourage the growth of certain mycobacteria, especially *Myco. kansasii.*

Hughes[4] confirmed Marks' comment that Pyruvic Egg Acid Medium is superior to glycerol, inasmuch as 5% of his isolated strains grew on pyruvate medium only.

Cruickshank[5] recommended the use of Pyruvic Acid Egg Medium for the isolation of human strains that are drug-resistant and difficult to grow.

References

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Introduction

Following studies of the constitution of egg media used for the culture of tubercle bacilli (Zaher, 1977[2]) it is possible to recommend a number of improvements. These will be presented in relation to current methods of preparing specimens for culture because a survey made by the authors has shown that laboratories not infrequently use media unsuitable for their inocula.

Materials and Methods

'Simple Method' for sputum culture (Marks[3])

This method was devised for laboratories with limited resources and is not as efficient as the full-scale method described next. Nevertheless it is widely used in Britain because it reduces work and hazard. Equal volumes of sputum and 4% NaOH are incubated at 35°C for 15 to 30 minutes, depending on the rate of liquefaction, and precisely 0.2ml is inoculated onto each slope. The medium now recommended for this method will be called *Acid Egg Medium,* No.1; it is prepared in a glycerol version (a) and a pyruvate version (b).

Acid Egg Medium No.1a: 6.3g KH_2PO_4, 0.3g $MgSO_4.7H_2O$, 12.0ml glycerol are dissolved in 600ml distilled water and autoclaved at 110°C for 15 minutes. To this solution the following are then added aseptically: 1100ml whole egg (mixed and strained through gauze), 32ml N HCl, 11ml malachite green (2% w/v) and 100 000 IU of sodium penicillin.

The medium is dispensed in volumes within the limits 4.5 to 5.0ml in 28ml bottles and inspissated by a single heating. The oven is brought to 75°C and held

between 75 and 85°C for 45 minutes. The high egg content is needed to provide a firm slope.

Acid Egg Medium No.1b: This is prepared as above, but the 12.0ml glycerol are replaced with 7.0g of sodium pyruvate.

Reference Laboratory method for sputum (Marks and Thomas[4])
Two ml of 4% NaOH are made up to 4ml with sputum or in the case of scanty specimens, with sputum and sterile water. After incubation for 15 to 30 minutes as above, the treatment bottle is held at an angle and 16ml of sterile water poured in from a 28ml (universal) container. Centrifugation and decanting follow and the deposit is divided between two slopes and a film. The medium recommended for this method will be called *Acid Egg Medium No.2;* it is also made in glycerol (a) and pyruvate (b) versions.

Acid Egg Medium No.2a contains 2.4g KH_2PO_4, 0.3g $MgSO_4.7H_20$, 12.0ml glycerol and 600ml distilled water. The ingredients are dissolved in the water autoclaved at 110°C for 15 minutes. To this solution the following are then added aseptically: 1000ml whole egg (mixed and strained through gauze), 40ml N HCl, 10ml malachite green (2% w/v) and 100 000 IU of sodium penicillin. The medium is distributed and inspissated as for *Acid Egg Medium No.1*. In the pyruvate version (2b) the 12.0ml glycerol are replaced by 7.0g sodium pyruvate.

Petroff's method and modifications
In the classical method of Petroff and in its modifications, sputum is incubated with an equal volume of 3% or 4% NaOH. The mixture is then neutralised, centrifuged and the deposit inoculated. Some workers centrifuge first, with or without preliminary dilution, and neutralise the deposit. The medium recommended for this method is called *Simplified Lowenstein-Jensen (L-J)* and it is made in glycerol and pyruvate versions.

Simplified L-J Medium This medium has the same formula as Acid Egg Medium No.2 except that the hydrochloric acid is omitted and the volume of malachite green solution is increased to 20ml.

Sulphuric acid treatment
Apart from sputum and the deposit of gastric contents, most specimens are best treated with sulphuric acid as described by Marks[5]. However, the Reference Laboratory has now reduced the maximum reagent concentration of 5% to 4% (v/v).

Treatment is followed by dilution, centrifugation and decanting which leaves a moderately acid deposit for inoculation. The medium recommended for this method is *Simplified L-J Medium.*

Results
The preliminary experiments which led to the improvements of medium formulation is cited elsewhere (Zaher 1977). However, the results of three final trials are presented here. The 'simple method' of sputum culture used to compare *Acid Egg Medium No.1* with the 'acid egg medium' originally devised for this method of culture. Glycerol and pyruvate versions of both media were used in parallel for 50 specimens selected as likely positives. The new formula gave earlier or heavier growth in 25 cases and the original medium in 3 cases, a significant advantage of the new medium ($P<0.001$); growth was equal in 14 cases and absent in the remainder.

The Reference Laboratory's method for sputum was used to compare *Acid Egg Medium No.2b* with the pyruvate version of the method's original 'buffered egg medium'. This trial was conducted on unselected routine specimens. The new formula gave earlier or heavier growth in 102 cases and the original medium in 23 cases, which is again a significant advantage to the new medium ($P<0.001$); growth was equal in 62 cases. All the media in these two trials were given the same low content of malachite green (approximately 0.0125%) as the object was to isolate the effect of phosphate concentration.

The third trial compared 0.0125% and 0.025% malachite green in *Acid Egg Medium No.2b* using unselected specimens. With the weaker dye, growth was earlier or heavier in 32 cases and with the stronger dye in 7 cases, a significant advantage to the lower concentration of malachite green in this medium ($P<0.001$); growth was equal in 26 cases.

Discussion
Four procedures for preparing specimens for culture are described above and these provide respectively inocula which are (1) highly alkaline, (2) moderately alkaline, (3) neutral or slightly acid (by over-shoot) and (4) moderately acid. Lowenstein-Jensen Medium and its simplified versions are suitable for the inocula in groups (3) and (4) which vary from neutral to moderately acid, but the pH of this medium rises to an unfavourable level with alkaline inocula. The media originally recommended for the methods providing alkaline inocula depended on phosphate buffer for control of the pH.

Experiments preliminary to the present work showed that the concentrations of phosphate used were detrimentally high. In the new media, phosphate has been partially replaced by HCl. Malachite green is more antibacterial at lower pH levels and the present work has confirmed that lower concentrations than usual should be used in acid egg medium.

The *Simplified L-J Medium* described above is based on earlier work aimed at simplifying Lowenstein-Jensen Medium (Marks and Thomas[4], Stonebrink (1961)[6]) and has not been subjected to further trials. Credit for the introduction of pyruvate medium is due to Stonebrink[6]. We advise that most types of specimen should be cultured on both glycerol and pyruvate medium as tubercle bacilli vary in their preference.

References

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THE OXOID DIP SLIDE

Code: DS

Kass[1] defined significant bacteriuria on the basis of colony counts of carefully collected, freshly voided, total specimens of urine obtained from populations of asymptomatic women. He found that, on the basis of the counts obtained, it was possible to distinguish between infected and contaminated urine. When the count was more than 10^5 organisms per ml in a single specimen, the probability was about 80 per cent that infection was present. If the observation was repeated in a further specimen, the probability of infection was greater than 90 per cent.

Since these original observations it has become apparent that when carefully collected, mid-stream urine samples are obtained from symptomatic patients, the colony counts required for the diagnosis of urinary tract infection are lower than those defined by Kass for asymptomatic populations[2,3]. A count of more than 10^2 organisms per ml can now be regarded as diagnostic in a woman with acute symptoms of urinary tract infection, while in men the diagnostic count is 10^3 organisms per ml. In most circumstances, however, the counts obtained will be greater than 10^5 organisms per ml.

OXOID DIP SLIDE REGRESSION SLOPE

Since the original observations by Kass many cultural and chemical methods have been devised to measure the bacterial population of urine.

All these methods depend on freshly voided mid-stream urine promptly delivered to the bacteriology laboratory for examination, either immediately or after brief refrigerated storage.

Mackey and Sandys[4] devised the method of filling a spoon with a solid medium and using this as a dip-inoculum transport medium, thus avoiding erroneous counts that may result from bacterial growth in the urine between collection and examination in the laboratory.

The 'Dip Slide' was a logical development of this method[5,6]. By use of a microscope slide coated on each side with a different culture medium larger areas of medium were available to obtain a semi-quantitative measure of the bacterial count.

Oxoid investigated the dip slide and concluded that though the basic principle was satisfactory, useful changes could be made in the design. The difficulty of handling microscope dip slides without contaminating the media, the relatively thin layers of the coating agar and the uneven draining of the urine on to a paper disc in contact with the bottom of the slide, were disadvantages not evident with the dip spoon.

The principle is simple. An agar-coated slide is provided, attached to the cap of a screw-capped container. By dipping the slide into freshly voided urine, reliable counts can be obtained. These show the number of organisms present in the urine at the time of voiding.

The method has the following advantages:

1 The Dip Slide technique is the simplest, most rapid and most reliable method for determining the presence or absence of urinary tract infection.
2 Counts on a Dip Slide have been shown to correlate closely with counts made by careful quantitative methods in the laboratory[6,8,9,10].
3 Since the slide is dipped into freshly voided urine, the counts obtained reflect the number of organisms present at the time the urine was passed. It is well-known that, as a result of its growth supporting properties, urine held for some time at room temperature before examination cannot be relied upon to yield reliable colony counts.
4 It is usually a simple matter for the practised eye, when the Dip Slide is used, to distinguish between urinary infection and contamination from other sources.
5 The time required by the medical laboratory scientist to examine each specimen is minimal. Screening of large numbers of specimens can be achieved almost at a glance.
6 The Dip Slide inoculation technique is sufficiently simple that it can be carried out at home by the patient.
7 The Dip Slide technique can be used equally for the examination of urine from infants and children.

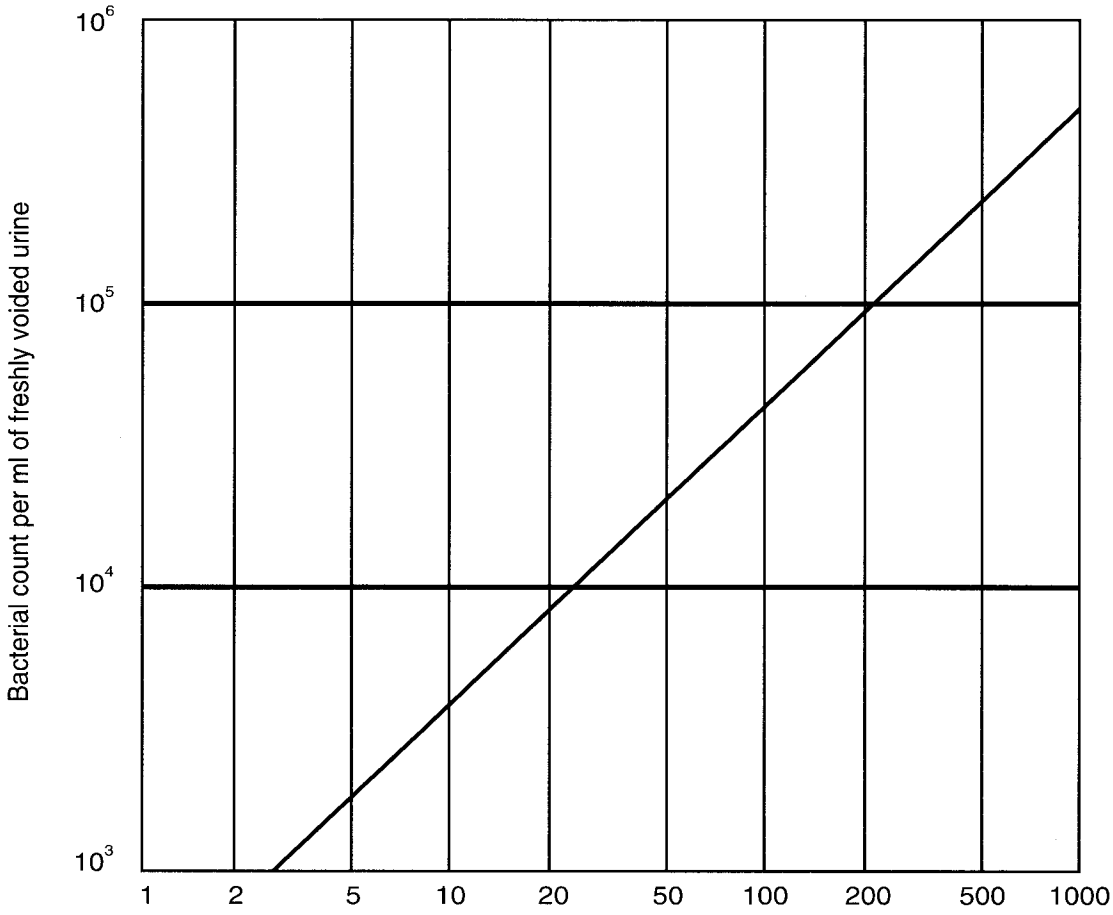
METHOD OF USE

The object is to wet both sides of the Oxoid Dip Slide with a mid-stream specimen of urine. This is done by collecting a mid-stream specimen in a sterile container and then dipping the slide into the urine. After dipping, the slide is held vertically for a moment to allow any surplus urine to drain from its lower end, after which the slide is returned into its sterile container.

Arniel[11,12] devised a 'dip-stream' technique that is particularly suitable for children but can equally be used with adults. The patient commences micturition and, when the stream is well-established, the Dip Slide is held into it sufficiently to wet each side thoroughly. The slide is then allowed to drain briefly and is returned into its sterile container. The Dip Slide dip-stream technique is completely reliable and inexpensive[6,7] and can be used for large-scale screening.

With both methods care must be taken to ensure that the agar surface is not touched and patients must be carefully instructed about the technique they should follow[13]. It is helpful if the vulva are held apart during the collection of mid-stream urine, but cleansing is not important. If it is done for any reason, antibacterial soaps and antiseptics must be avoided.

The patient's name and details of the date and time of collection are noted on the label and the container with the Dip Slide is either posted or taken to the



laboratory for incubation and examination. In the case of *E. coli* infections – which constitute the majority of urinary tract infections – incubation at room temperature for 24 hours is sufficient to produce readable colonies[8]. Samples sent by post are, therefore, likely to be ready for examination on receipt by the laboratory. Apparently negative Dip Slides should, of course, be incubated for 18 hours before they are discarded.

THE SLIDE

The Oxoid Dip Slide is made of plastic and is provided with raised edges to ensure an even thickness of medium. The Slide has a moulded grid to simplify colony counting, and a terminal spike to ensure adequate draining of the urine. The dimensions of the slide are 19mm x 50mm (950mm^2 agar surface area). The Slide is enclosed in a light-weight plastic bottle which protects the slide but allows easy observation of bacterial growth.

THE MEDIA

The slide is coated with MacConkey Agar on one side and C.L.E.D. (Cystine Lactose Electrolyte-Deficient) Medium on the other.

The MacConkey Agar is provided because of its well-known diagnostic characteristics for organisms that grow on bile salt media.

The C.L.E.D. Medium is made to the formula described by Mackey and Sandys[14]. It is specifically recommended for urine bacteriology, because it supports the growth of all common urinary pathogens and gives colonial differentiation and clear diagnostic characteristics. Important contaminants, such as diptheroids, lactobacilli and micrococci are also clearly shown and give a good indication of the degree of contamination. Since C.L.E.D. Medium is electrolyte-deficient, it prevents the swarming of *Proteus* species. Mixed growths that are typical of contamination rather than true infection are, therefore, easily recognised. Mixed cultures of significant organisms can easily be picked and examined after sub-culture.

INTERPRETATION OF DIP SLIDE RESULTS

After incubation, the slide is examined for evidence of bacterial growth. On the reliable assumption that each colony represents a single bacterial cell in the urine sample, there is a correlation between the number of bacteria in the specimen and the number of colonies on the slide.

Until recently it was assumed that contaminants were present in counts of less than 10^4 organisms per ml, while in true infection the counts exceed 10^5 per ml. In calibration experiments 10^4 organisms per ml give about 30 colonies on the slide, while 10^5 organisms give about 300 colonies. The Dip Slide, therefore, provides maximum discrimination over the usual range of bacterial counts found in urinary tract infections.

When the results of the Dip Slide test are read, the colony count and purity of growth should be assessed from the C.L.E.D. Medium, which is initially green, and **not** from the MacConkey Medium which is initially red. The MacConkey Medium is provided only to assist in the identification of the organisms present. However, since in community-acquired infection generally and in children the overwhelming majority of urinary tract infection is due to *E. coli*, reading of the MacConkey Medium alone gives a low level of false-positive results.

When, as is often the case, the bacterial count is very high, the surface of the medium may be covered by a confluent of growth that is easily overlooked unless the slide is examined in reflected light and compared with an uninoculated slide. Confluent growth shows up as the absence of a reflection and by this means the observer should also be able to pick out very small colonies that would otherwise be overlooked.

The bacterial content of urine is normally expressed as follows:

Positive – more than 100,000 bacteria per 1ml of urine (10^5 organisms/ml)

Doubtful – between 10,000 and 100,000 bacteria per 1ml of urine (10^4–10^5 organisms/ml)

Normal – less than 10,000 bacteria per 1ml of urine (10^4 organisms/ml)

The above figures apply to a single type of organism grown on an appropriate medium such as C.L.E.D.

While these figures represent useful ranges of bacterial numbers, it should be recalled that under some circumstances counts as low as 10^2 organisms per ml may be significant.

If the growth obtained consists of more than one type, this almost certainly represents contamination and, depending on the circumstances, the test may have to be repeated.

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5
BIOCHEMICAL REAGENTS AND DIAGNOSTIC DISCS

November 1998

BIOCHEMICAL REAGENTS AND DIAGNOSTIC DISCS

BIOCHEMICAL REAGENTS

Code: BR

These products are a diverse mixture of biological and chemical reagents used in microbiology. There are no general rules for these reagents and the storage conditions given for each product should be followed. Store as directed and use in strict order of the batch/lot number to ensure proper stock rotation. Identification reagents should be checked with known strains of positive and negative organisms to monitor the performance of the reagents.

Biochemical reagents are classified under the following general headings:

Biological Indicator
Bacillus stearothermophilus Spore Strips BR23

Buffer Salts
Barbitone Complement Fixation Diluent BR16*
Phosphate Buffered Saline (Dulbecco A) BR14a

Culture Medium Indicator
Basic fuchsin (for use with Endo Agar Base CM479) BR50*

Identification Tests
Beta-lactamase Detection Papers BR41
Beta-lactamase Detection Sticks BR66
MUG Reagent BR71
Oxidase Detection Sticks BR64

Ringer-Saline Tablets
Calgon Ringer Solution BR49
Ringer Solution BR52
Thiosulphate Ringer BR48
Saline Tablets BR53

***Hazchem precautions**
Barbitione is a registered narcotic drug in the UK and many other countries. The strict regulations which apply to this drug about licences to send and receive the product, about storage conditions and record keeping must be observed by sender and receiver.

Basic fuchsin is closely related to known carcinogenic compounds. It should be handled with care to avoid inhaling the powder or staining the skin.

BARBITONE CFT DILUENT TABLETS

Code: BR16

Formula	**gm/litre**
Barbitone	0.575
Sodium chloride	8.5
Magnesium chloride	0.168
Calcium chloride	0.028
Barbitone soluble	0.185
pH 7.2 + 0.2	

Directions
Dissolve 1 tablet in 100ml of warm distilled water.

Description
Complement fixation tests are extensively employed in the diagnosis of many different diseases, including infections due to viruses as well as bacteria.

These tablets are intended for the simple and convenient preparation of the special diluent for use in complement fixation tests. This diluent, a modification of that described by Whillans[1], is similar to the diluent employed by Mayer *et al*[2]. It is superior to the physiological saline formerly used because it contains calcium and magnesium, and is of the correct pH for optimum results.

Mayer *et al*.[2] investigated the effect of cations on the haemolytic function of complement, by the addition of a number of substances to the veronal buffered saline base. They were able to conclude:

i Some divalent cations, especially Mg^{++}, are essential for the haemolytic action of complement.

ii The haemolytic system does not contain sufficient Mg^{++} for optimal haemolytic activity, so that a marked enhancement can be obtained by the addition of extra Mg^{++}. The enhancing effect of tissue fluids can be ascribed to their contribution of Mg^{++}.

iii The anticomplementary effect of some substances can be overcome by the addition of extra Mg^{++}.

iv Ca++ may also be essential to the haemolytic process but its action is much less pronounced than that of Mg^{++}.

The use of Oxoid Complement Fixation Test Diluent Tablets in a description of complement fixation tests for virus diseases was recommended[3,4].

Precaution
Barbitone is a registered narcotic drug and subject to control regulations on transport, storage and records. See Hazchem precaution (page 2–7).

References

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(BASIC) FUCHSIN

Code: BR50

Description
This dye has been selected for use in Endo Agar Base CM479. It should be dissolved at 10% w/v in 95% ethyl alcohol. For each litre of medium add the appropriate volume of alcoholic solution shown on the label (usually 8ml per litre). The magenta dyes are closely related to known carcinogenic substances; therefore basic fuchsin should be handled with care, avoiding inhaling the powder or staining the skin with dye.

MUG REAGENT

Code: BR71

A fluorescent agent for the detection of Escherichia coli.

Vial content

4-methylumbelliferyl-β-D-glucuronide	50mg

Directions
Add 2ml of distilled water to a vial and invert gently until completely dissolved. Add the vial contents to the following volumes of suggested media, before sterilisation.

Medium	*Final conc. of MUG per litre*	*Number of vials per litre*
Violet Red Bile Agar CM107	100mg	2
MacConkey Agar No. 3 CM115	100mg	2
Brilliant Green Bile (2%) Broth CM31	50mg	1
MacConkey Broth Purple CM5a	50mg	1
Lauryl Tryptose Broth CM451	50mg	1

Other media can be used, consult Oxoid for further advice.

Description
Oxoid MUG reagent is a lyophilised presentation of the substrate 4-methylumbelliferyl-β-D-glucuronide in 50mg quantities. The incorporation of MUG reagent into culture media is reported to improve the sensitivity and specificity of *Esch. coli* detection[1,2,3,4,5]. This improved sensitivity is mainly due to the detection of anaerogenic strains of *Esch. coli* when present in mixed cultures. The sensitivity reported for various media varies from 59% to 85.8%[6].

MUG reagent is cleaved by the enzyme glucuronidase to release an end product 4-methylumbelliferone which produces a visible green/blue fluorescence under long wave ultra-violet light (366nm). The addition of MUG reagent to culture media provides another criterion by which to determine the presence of *Esch. coli* in food and environmental samples.

Alkaline pH increases the intensity of fluorescence. Maddocks and Greenan[7] adjusted the pH of their cultures with sodium hydroxide to maximise light output in their investigations of MUG hydrolysis as an alternative to conventional biochemical tests for identifying bacteria. The acidification of the agar surrounding *E. coli* colonies on lactose-based media diminishes the discrimination of MUG-hydrolysing colonies[8]. Freir and Hartman[9] exposed membrane filter cultures to ammonia vapour to enhance fluorescence. A phosphate-buffered MUG agar was used by Entis and Boleszczuk to minimise pH fall in an improved 24 hour hydrophobic grid membrane filter method for coliform and *E. coli* enumeration[10].

MUG has been included in media used with membrane filters in methods for enumeration of *E. coli* in foods, water and sewage[11,12]. Conditions that affect the fluorescence intensity of MUG were investigated by Villari, Iannuzzo and Torre and recommendations made for its optimum use[13].

Technique
Follow the method and procedure relevant to the sample and the selected medium. Uninoculated tubes or agar plates should be used as controls. (See Precaution.)

After incubation detect glucuronidase activity by examining the microbial growth under UV light (366nm).

The presence of blue/green fluorescence indicates glucuronidase activity.

Report fluorescence as presumptive presence of *Esch. coli* and confirm by further biochemical tests.

Storage and stability
Should be stored at 2–8°C. When stored as directed the unopened vial is stable until the expiry date on the label.

Quality control
Positive control:
Escherichia coli ATCC® 25922

Negative control:
Proteus mirabilis ATCC® 10975

Precautions
The presence of endogenous glucuronidase in shellfish samples may result in false positive fluorescence. Test tubes used in the MPN method should be checked under UV light to ensure the glass does not fluoresce. To avoid false positive flourescence the source of long wave UV light must not exceed 6 watts.

References

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PHOSPHATE BUFFERED SALINE

Code: BR14a (Dulbecco 'A' Tablets)

Formula	gm/litre
Sodium chloride	8.0
Potassium chloride	0.2
Disodium hydrogen phosphate	1.15'
Potassium dihydrogen phosphate	0.2
pH 7.3	

Directions
Dissolve 10 tablets in 1 litre of distilled water and autoclave for 10 minutes at 115°C. The solution will be quite free from insoluble matter.

Description
This balanced salt solution is issued to meet the requirements of those tissue culture workers who use the Dulbecco Solution with and without calcium and magnesium. Oxoid Dulbecco 'A' Solution corresponds to the original formulation of Dulbecco and Vogt[1] except that calcium and magnesium are omitted and may be added separately.

An example of the use of Solution 'A' is in the suspension of cells from a monolayer culture[2]. Cells growing in monolayer on a glass surface may be brought into suspension by a number of methods, including the application of chelating agents such as Versene (diamino-ethane-tetra-acetic acid) or its salts. Chelating agents bind divalent cations, such as calcium and magnesium ions, and it is probably this effect which causes the cells to become detached from the glass. Dulbecco 'A' Solution is therefore used to rinse the sheet of cells free from growth medium before application of the chelating agent – this latter solution may consist of Dulbecco 'A' Solution containing 0.02% w/v of sodium versenate and 0.02% w/v of glucose.

Molarity
The molarity of a solution of phosphate-buffered saline is expressed as the number of moles of solute in 1 litre and is associated with each species in a mixture.

Molarity figures given are for the individual components of the formula.

Sodium choloride	0.16m
Potassium chloride	0.003m
Disodium hydrogen phosphate	0.008m
Potassium dihydrogen phosphate	0.001m
Total	0.172

References
1 Dulbecco and Vogt (1954) *J. Exp. Med.* 99. 167–182.
2 Paul J. (1965) *'Cell and Tissue Culture' 3rd ed, Livingstone Ltd., London.*

BETA-LACTAMASE DETECTION PAPERS

Code: BR41

Description
With the emergence of ampicillin resistance in *Haemophilus influenzae* and *Neisseria gonorrhoeae*, it has become desirable to have a rapid method for detecting it. This test detects the enzyme beta-lactamase in those strains of *H. influenzae* and *N. gonorrhoeae* which produce it. The reagents are stable and the test is rapid – not requiring incubation.

Technique
Place one drop of distilled water on a clean microscope slide and cover with a test paper so that the paper is moist but not over-saturated.

Using a wire loop or a wooden stick pick one or more colonies and streak onto the dampened strip.

The presence of a beta-lactamase-producing strain is indicated by the streaked portion of the strip changing colour from violet to yellow after 5 minutes approximately. Known positive and negative controls should be applied to each strip.

The colour remains stable after the papers have dried.

Please note that the method is only suitable for detection of beta-lactamase which becomes dissociated from the cell, or, if remaining cell associated, is in a readily accessible site. It is not suitable for species where induction is necessary for enzyme elaboration or where the enzyme remains strongly cell-bound.

Discussion
Conventional diffusion tests for antibiotic susceptibility do not usually yield a result in less than 48 hours from the time the specimen is collected and are consequently often not sufficiently rapid for detection of ampicillin resistance in *H. influenzae*. Furthermore, because of the marked influence of both inoculum size and medium composition on the apparent susceptibility of *H. influenzae*, results obtainable from such tests provide a less reliable indication of antibiotic susceptibility *in vivo* than for most other species encountered[5,6,7]. Ampicillin resistance in *H. influenzae* and *N. gonorrhoeae* is due to an acquired capacity to elaborate a beta-lactamase. Intrinsic resistance is uncommon and induction is not required[8,9]. The enzyme remains associated with the cell but occupies a readily accessible site and for this reason is rapidly activated when a dense suspension of intact bacteria is mixed with penicillins. Tests have shown that the beta-lactamase of cell suspensions is relatively stable and that rapid hydrolysis of penicillins occurs at 25°C over a broad pH range[10].

Several tests have been described which allow recognition of bacteria that produce beta-lactamase as soon as growth is apparent in isolation media[11,12,13,14]. They are not entirely satisfactory because the methods

utilise unstable reagents with a short shelf life. Also, because they require incubation, they are slow.

In contrast the method used for testing for beta-lactamase with Oxoid Detection Papers utilises a system with a greater inate stability. The test is also fast; a result may be obtained in 3–5 minutes from a single colony picked as soon as there is visible growth on the culture plate.

Oxoid Beta-Lactamase Detection Papers are impregnated with benzyl-penicillin and a pH indicator[15]. The method utilises the resulting drop in pH of benzyl-penicillin upon hydrolysis by a beta-lactamase to record a change in colour of the indicator dye from violet to yellow.

The test may be used for routine surveillance for beta-lactamase-producing strains of *H. influenzae* and *N. gonorrhoeae*, giving full agreement with the chromogenic cephalosporin substrate method[15].

The method is not suitable for detection of beta-lactamase enzymes from other species where induction is necessary for enzyme elaboration or where the enzyme remains cell-bound.

Conventional antibiotic disc sensitivity testing of *H. influenzae* with ampicillin under adequately controlled conditions will ensure the detection of rare ampicillin-resistant non-beta-lactamase-producing strains.

References

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BETA-LACTAMASE IDENTIFICATION STICKS

Code: BR66

Oxoid Identification Sticks, Beta-Lactamase are used for the detection of beta-lactamases produced by Gram-positive and Gram-negative bacteria. The tips of the sticks are impregnated with Nitrocefin, a chromogenic cephalosporin (Glaxo Research 87/312). The sticks are convenient to use and overcome the necessity for preparing fresh reagents daily.

Formula

One end of each stick is impregnated with a solution of Nitrocefin, phosphate buffer and dimethylsulphoxide. The opposite end is coloured black to identify the correct end for handling. The colour also identifies the product.

General Introduction and Intended Uses

Nitrocefin is the chromogenic cephalosporin developed by Glaxo Research Limited coded 87/312; 3 – (2,4 dinitrostyrl) – 6R, 7R) – 7 – (2 thienylacetamido)-ceph-3-em-4 carboxylic acid, E-isomer. This compound exhibits a rapid distinctive colour change from yellow to red as the amide bond in the beta-lactam ring is hydrolysed by a beta-lactamase (E.C.3.5.2.6.); it is sensitive to hydrolysis by all known lactamases produced by Gram-positive and Gram-negative bacteria.

Demonstration of Beta-Lactamase Activity by Bacterial Cells

Nitrocefin hydrolysis has been found to be highly efficient in detecting beta-lactamase producing isolates of *Neisseria gonorrhoeae*,[1,2,3] *Haemophilus influenzae*,[2,4,5,6] staphylococci[5,6] and bacteroides[7,8,9,10,11]. It should be emphasised that the efficiency of Nitrocefin tests in predicting the beta-lactam susceptibilities of other micro-organisms is at present unproven. In a positive reaction the beta-lactamase enzymes hydrolyse the amide bond in the beta-lactam ring. This is detected by a colour change on the end of the stick from yellow to pink/red.

Lactamase Induction

It should be noted that some organisms will not exhibit lactamase unless the enzyme has been induced by exposure to a beta-lactam antimicrobial. In such circumstances the organism should be tested from growth adjacent to beta-lactam antimicrobial discs or from agar containing beta-lactams.

Nitrocefin hydrolysis will give a rapid indication of beta-lactamase activity and the result so obtained will, in most cases, predict the outcome of susceptibility tests with these antimicrobials. However, it should not entirely replace conventional susceptibility testing as other factors also influence the results of such tests.

Technique

1 Remove the container from the freezer and allow it to reach room temperature.

2 Select a well separated representative colony from the primary isolation medium.

3 Remove one stick (colour coded black) from the container and holding the coloured end, touch the

colony with the impregnated tip of the stick, **rotate the stick**, picking up a small mass of cells.

4 Place the inoculated tip of the stick between the lid and the base of the inverted plate.

5 **The reaction requires moisture. The inoculated tip of the stick should be placed in the moisture condensate in the lid. If condensate is not available in the inverted lid add one or two drops of distilled water to the lid and moisten the tip of the stick.**

6 **Examine the reagent impregnated tip of the stick for up to five minutes and, if negative re-examine after fifteen minutes.**

Note: Some staphylococci may take up to 1 hour before reaction shows a colour change.

7 A positive reaction is shown by the development of a pink/red colour. No colour change is observed with organisms that do not produce beta-lactamase. To ensure correct reading the colour of the stick should be compared to an unused stick.

Caution
Organisms producing pigmented colonies i.e. *Staph. citreus*, may give false positive results. It is recommended therefore that when pigmented colonies are to be tested Nitrocefin in solution – code SR112, should be used.

Quality Control
Reference strains should be tested to control the product at appropriate intervals.

Positive control:
Beta-lactamase producing staphylococcus

Negative controls:
Non beta-lactamase producing staphylococcus

Storage Temperature
Store below –10°C.

References

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OXIDASE IDENTIFICATION STICKS

Code: BR64

A convenient and stable presentation of oxidase reagent for the detection of oxidase-positive bacteria.

The enzyme cytochrome oxidase is produced by many organisms including *Neisseria* and *Pseudomonas* species and the 'Oxidase Test' is an important and commonly used reaction for the screening and presumptive identification of microbial cultures. Unfortunately, the reagent used is unstable in solution and needs frequent preparation for reliable results to be obtained.

Oxidase Identification Sticks utilise a dry reagent specially stabilised to give it a long life. They therefore overcome the necessity for daily preparation of fresh reagent and are very convenient to use.

Formula
The tip of each stick is impregnated with a solution of N,N-dimethyl-p-phenylenediamine oxalate, ascorbic acid and α-napthol. The other end is coloured red for identification and to ensure that the correct end is held.

Description
The Oxidase test is an important differential procedure which should be performed on all Gram-negative bacteria that are to be identified. The Oxidase reaction, based upon the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and α-napthol was introduced by Gordon and McLeod[1] to aid in the identification of gonococci. Its wider use originated with the test devised by Kovacs[2], to distinguish *Pseudomonas* species from enteric bacteria. Kovacs smeared bacterial growth on filter-paper impregnated with 1% w/v aqueous tetramethyl-p-phenylenediamine dihydrochloride solution. Steel[3] found Kovacs' method too sensitive, with some staphylococci giving weak or delayed reactions. More useful results were obtained by the method described by Gaby and Hadley[4] using N,N-dimethyl-p-phenylenediamine oxalate where all staphylococci were oxidase negative.

Barry and Bernsohn[5] confirmed the observations of Carpenter et al[6] that dried crystals of the oxalate salt dimethyl-p-phenylenediamine have a longer shelf life than the tetra-methyl-p-phenylenediamine dihydrochloride. The loss of activity of the oxidase reagents is caused by autoxidation which may be retarded by the addition of 0.1% ascorbic acid[3]. Filter paper strips impregnated with the oxidase reagents and their use have been described by Rogers[7] and by Barry and Bernsohn[5]. However, the Oxoid oxidase stick, impregnated with oxidase reagents described by Gaby and Hadley[4], is a much more convenient technique to use. The colony under test is touched with the impregnated end of the stick so that some microbial mass is picked from the colony. The use of the stick also overcomes the problems of iron oxidation of the reagent associated with nichrome wire loops.

In a positive reaction the enzyme cytochrome oxidase combines with N,N-dimethyl-p-phenylenediamine oxalate and α-napthol to form the dye indophenol blue.

Technique
1 Remove the container from the refrigerator and allow it to stand for 5 minutes at room temperature.
2 Choose a well separated representative colony on the primary isolation medium.
3 Remove one stick (colour coded red) from the container and holding it by the coloured end, touch the colony with the impregnated end of the stick. **Rotate the stick**, picking off a small mass of cells.
4 Place the stick between the lid and the base of the inverted plate.
5 Examine the impregnated end of the stick after 30 seconds. If a colour change has not occurred examine again after 3 minutes.
6 A positive reaction is shown by the development of a blue-purple colour. No colour change is observed with organisms that are oxidase negative.

Main Uses
To differentiate *Neisseria* (oxidase positive) from other Gram-negative diplococci.

To differentiate strains of *Pseudomonas* species (pigmented and non-pigmented) from other enteric organisms.

To differentiate *Aeromonas hydrophila* (oxidase positive) from *Escherichia coli* (negative).

To differentiate *Plesiomonas shigelloides* (oxidase positive) from *Shigella sonnei* (negative).

Quality Control
Reference strains should be tested to control the product at appropriate intervals.

Positive Control:
Pseudomonas aeruginosa ATCC® 27853

Negative Control:
Staphylococcus aureus ATCC® 25923

Precaution
Cytochrome oxidase production may be inhibited by acid production and false negative reactions may be given by *Vibrio*, *Aeromonas* and *Plesiomonas* species when growing on a medium containing a fermentable carbohydrate e.g. McConkey Agar and TCBS Agar. However, well isolated non-fermenting colonies can be tested. Colonies picked from media containing nitrate may give unreliable results.

Storage Temperature
Store at 2 to 8°C.

References

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3 Steel K. J. (1962) *J. Appl. Bact. 25. 445–447.*
4 Gaby W. L. and Hadley C. (1957) *J. Bact. 74. 356–358.*
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'CALGON' RINGER TABLETS

Code: BR49

Formula	gm/litre
Sodium chloride	2.25
Potassium chloride	0.105
Calcium chloride $6H_2O$	0.12
Sodium bicarbonate	0.05
Sodium hexametaphosphate ('Calgon')	10.0
pH 7.0	

One tablet makes 10ml of quarter-strength Ringer Solution containing 1% of 'Calgon'. (Sodium hexametaphosphate.)

Directions
To prepare a solution containing 1% sodium hexametaphosphate in quarter-strength Ringer Solution, dissolve 1 tablet in 10ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Higgins[1] showed that bacteriological swabs prepared from calcium alginate wool will dissolve completely in 'Calgon' Ringer Solution, thus releasing all the organisms taken up on the swab and giving a more accurate quantitative recovery. She concluded that the use of this material in place of cotton-wool for the preparation of swabs, seemed justified in quantitative work since the recovery of organisms was much greater.

This principle was successfully applied to the bacteriological examination of tableware[2] and also of crockery and kitchen utensils[3].

Trimarchi[4] found that calcium alginate swabs were superior to raw cotton swabs for the bacteriological examination of eating utensils.

Technique
Prepare swabs, using approximately 25–50 milligrams of alginate wool to each wooden applicator. Sterilise, in plugged tubes, by autoclaving at 121°C for 15 minutes. After swabbing, aseptically break off the swab end and drop into a screw-top bottle containing 10ml of sterile 'Calgon' Ringer Solution. Close the bottle and shake vigorously for 10–30 minutes to dissolve the alginate wool completely. The suspension may then be examined quantitatively or qualitatively in the usual manner.

References

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4 Trimarchi G. (1959) *Igiene Moderna 52. 95–111.*

RINGER SOLUTION – 1/4 STRENGTH RINGER SOLUTION TABLETS

Code: BR52

Formula	gm/litre
Sodium chloride	2.25
Potassium chloride	0.105
Calcium chloride $6H_2O$	0.12
Sodium bicarbonate	0.05
pH 7.0	

One tablet makes 500ml of quarter-strength Ringer solution.

Directions
To prepare quarter-strength Ringer Solution, dissolve 1 tablet in 500ml of distilled water. Sterilise by autoclaving at 121°C for 15 minutes.

Note
It is our experience that providing the quality of the water and glassware used in preparation conform to the specifications laid out in the Oxoid Manual (Section 2 page 4), under ''General Guide to the use of Oxoid culture media''; a pH of 7.0 $\pm$ 0.2 prior to autoclaving can be considered confirmation of the integrity of the product. Variation of pH out of specification after autoclaving is most likely to be caused by factors originating from sources other than the product e.g. water, glassware and autoclaving.

Description
These tablets are prepared according to the formula in the Dept. of Health & Social Security publication Memo. 139/Foods[1]. They dissolve readily in water to give a solution which does not precipitate when sterilised by autoclaving.

The sterile solution is used as an isotonic diluent for both bacterial cells and bacteriological specimens, or as a rinse during the bacteriological examination of plant and apparatus.

Technique
Oxoid quarter-strength Ringer Solution may be used for the decimal dilution of milk and dairy product samples, e.g. prior to inoculation into MacConkey Broth for the presumptive coliform test, the maceration and suspension of solid food and other specimens before bacteriological examination; the preparation of suspensions and serial dilutions from pure cultures of bacteria; the examination of dairy plant and apparatus by the swab or rinse method[2].

See also Thiosulphate Ringer Tablets BR48 and 'Calgon' Ringer Tablets BR49.

References

1 Dept. of Health & Social Security (1937) *Memo. 139/Foods.*
2 Davis J. G. (1956) *'Laboratory Control of Dairy Plant'. Dairy lndustries Ltd., London.*

THIOSULPHATE RINGER TABLETS

Code: BR48

Formula	gm/litre
Sodium chloride	2.15
Potassium chloride	0.075
Calcium chloride $6H_2O$	0.12
Sodium thiosulphate $5H_2O$	0.5
pH 6.6	

One tablet makes 500ml of quarter-strength Ringer containing 0.05% of sodium thiosulphate.

Directions
To prepare quarter-strength Ringer Solution containing thiosulphate, dissolve 1 tablet in 500ml distilled water. Sterilise by autoclaving at 121°C for 15 minutes.

Description
Oxoid Thiosulphate Ringer Tablets are formulated to provide, after the addition of distilled water, a Ringer Solution (British Pharmacopoeia[1]) containing 0.05% of sodium thiosulphate hydrate. Use of the B.P. Ringer instead of the Ministry of Health Ringer ensures chemical stability without altering the physiological properties of the diluent. The purpose of the sodium thiosulphate is to neutralise residual chlorine present in bacteriological samples and rinses as a result of the previous use of gaseous chlorine or chlorine-containing disinfectants. The thiosulphate immediately terminates the bactericidal action of the chlorine in the sample – so that subsequent bacteriological examination more accurately indicates the pollution of the sample at the time of collection. Sterile Thiosulphate Ringer Solution is consequently employed for rinse water samples taken for sanitary tests (from equipment and pipe lines, etc.) where a chlorine-containing cleansing fluid has been used. Davis used a Ringer Solution with added sodium thiosulphate, as a rinse after the use of sodium hypochlorite[2]. Egdell et al.[3] employed a Thiosulphate Ringer Solution in their bacteriological studies of immersion cleaned milking equipment.

Technique
100ml of prepared solution will completely neutralise 7mg of chlorine. Oxoid Thiosulphate Ringer Solution may be used for assessing the cleanliness and sterility of dairy plant, after hypochlorite solution has been used for disinfection purposes (see Davis[2]). The methods described by Davis rely on swabbing or rinsing of apparatus or milk bottles. In reference to the swab method, see 'Calgon' Ringer Tablets.

References

1 British Pharmacopoeia (1953) *p 501.*
2 Davis J. G. (1956) *'Laboratory Control of Dairy Plant'. Dairy Industries Ltd., London.*
3 Egdell J. W., Lomax K. L., Adams R. P. and Aitken M. J. (1958) *J. Appl. Bact. 21(1). 109–117.*

SALINE TABLETS
(FOR LABORATORY USE ONLY)

Code: BR53

Dissolve 1 tablet in 500ml of distilled water in order to obtain 0.85% ('normal', physiological, or isotonic) saline.

DIAGNOSTIC DISCS

Code: DD

These products are, as the name implies, 6mm paper discs impregnated with compounds which aid the identification of micro-organisms when placed on agar surfaces or into suspensions of organisms.

Storage
All DD products should be stored at 2–8°C **except** V factor discs DD4 and XV factor discs DD5 which must be stored at –20°C to preserve the labile co-enzyme[1].

Discs should not be used beyond the expiry date shown on the label and containers should be used in strict batch/lot number order to ensure good stock rotation.

Moisture Protection
Cartridges of discs should be kept in moisture-proof containers with desiccant sachets and allowed to reach room temperature before opening. Discs in cartridges are protected from moisture but care should be taken not to open them before they have reached room temperature. This is especially important when cartridges are taken from –20°C storage.

Quality Control
Routine tests should be made using known strains of positive and negative-reaction organisms to confirm the activity of the discs and to control the other factors in the test.

ANAEROBIC GRAM NEGATIVE BACTERIA

'AN-IDENT' DISCS

Code: DD6

To provide a rapid and simple method for presumptive identification of anaerobic gram negative bacteria.

Disc Contents
The set consists of one cartridge of each of the antibiotic discs packed with a desiccant sachet in a securitainer. Each cartridge contains 50 discs. 'An-ident' discs should be stored according to the instructions on the container.

Antibiotic levels on the discs are as follows:

		Coded
Erythromycin	60μg	E DD
Rifampicin	15μg	RD DD
Colistin	10μg	CT DD
Penicillin	2 IU	P DD
Kanamycin	1000μg	K DD
Vancomycin	5μg	VA DD

The set should be arranged in the dispenser in the above order to coincide with the order given in the identification table. It is primarily designed to allow easy identification of *Bacteroides fragilis*, the species most commonly isolated in the routine hospital laboratory.

Description
'An-ident' discs provide a rapid and simple method for presumptive identification of anaerobic Gram negative bacteria. ***They are not suitable for antibiotic susceptibility testing to assess the clinical effectiveness of the various antibiotics.***

Sutter and Finegold[1] proposed a method for the identification of Gram negative anaerobic bacilli based on differences in response to a number of antibiotics. Using carefully chosen levels of colistin, erythromycin, kanamycin, neomycin, penicillin and rifampicin with disc diffusion sensitivity testing techniques they showed that these bacteria could be placed fairly consistently into five groups. Other simple biochemical tests and cultural characteristics were used for further identification. In later work summarised by Finegold[2] 5μg vancomycin discs were substituted for neomycin.

The Oxoid 'An-ident' set makes available these discs in a cartridge presentation suitable for use either in the Oxoid Dispenser or with Oxoid Ejectors. Ejectors can be used independently of the dispenser.

Technique
The technique as recommended by Leigh and Simmons[3] is as follows:
An actively growing culture in Thioglycollate Medium CM173 or Schaedler Broth CM497 is used as an inoculum. Using a sterile swab, spread the organism uniformly across the surface of a blood agar plate. Alternatively a flood-plate may be prepared from a suspension of several colonies in Thioglycollate Broth.

Discs of each antibiotic are then applied and the plate incubated anaerobically at 35°C for 24–48 hours. The Oxoid Gas Generating Kit provides a convenient way of achieving anaerobiosis and ensuring that adequate levels of CO_2 are present in the anaerobic jar. Strains of *Bacteriodes fragilis, Fusobacterium fusiforme, Sphaerophorus necrophorus* and *S. varius* are used as controls. The diameters of inhibition zones are measured in millimetres; less than 10mm is considered resistant; equal to or greater than 10mm considered sensitive.

Quality Control
See under Technique.

References

1 Sutter V. L. and Finegold S. M. (1971) *Applied Microbiology 21. 13–20.*
2 Finegold S. M. (1977) *Paper presented at the IMLS meeting, Liverpool, August 1977.*
3 Leigh D. A. and Simmons K. (1977) *J. Clin. Path. 30. 991–992.*

ANAEROBIC GRAM POSITIVE COCCI

SPS DISCS

Code: DD16

Sodium polyanethol sulphonate discs for the presumptive identification of Peptostreptococcus anaerobius.

Contents
1 cartridge. Each contains 50 discs.

Disc Content
SPS 1mg

Description
Wideman et al.[1] reported that *Peptostreptococcus anaerobius* may account for one-fifth to one-third of all Gram-positive cocci encountered in clinical specimens and confirmed that all strains of *Peptostreptococcus anaerobius* are totally inhibited by sodium polyanethol sulphonate (SPS) as described previously by Graves et al.[2]. It has been recommended[1] that the SPS disc method should be used as a rapid and simple method for the presumptive identification of *Peptostreptococcus anaerobius*. By this method all strains of *Peptostreptococcus anaerobius* give inhibition zones of 12 to 30mm. *Peptostreptococcus micros* and *Peptococcus prevotii* may give zones that overlap the zone size range obtained with *Peptostreptococcus anaerobius*.

SPS Disc Sensitivity of Clinical Isolates[1]

Organism	*SPS Disc Inhibition Zone Diameter (Range mm)*
Peptostreptococcus anaerobius	18–27
P. micros	6–12[a]
P. parvulus	No zone
P. productus	No zone
Peptococcus asaccharolyticus	No zone
P. magnus	No zone[b]
P. prevotii	6/16[c]
P. saccharolyticus	No zone
P. variabilis	No zone
Peptococcus species	No zone
Acidaminococcus fermentans	No zone
'Gaffkya anaerobia'	No zone
Streptococcus intermedius	No zone
S. morbillorum	No zone
Streptococcus species	No zone
Veillonella alcalescens	No zone
V. parvula	No zone

[a]Two isolates had zone diameters of 10mm and one isolate had a 12mm zone diameter.
[b]One isolate had a 17mm zone of diminished growth.
[c]One isolate had a zone of 16mm.

Technique
1 Prepare Wilkins-Chalgren Anaerobe Agar CM619 plates as directed.
2 Adjust the 18–24 hour culture of the test organism to give 0.5 of the McFarland No.1 nephelometer standard.
3 Evenly inoculate the surface of a Wilkins-Chalgren Anaerobe Agar plate with the culture under test.
4 Place one DD16 on each plate.
5 Incubate anaerobically at 35°C for 48 hours.
6 Observe zones of inhibition.

Quality Control
Use known strains of *P. anaerobius* to monitor the performance of the discs.

References

1 Wideman P. A., Vargo V. L., Citronbaum D. and Finegold S. M. (1976) *J. Clin. Micro. 4. 330–333.*
2 Graves M. H., Morello J. A. and Kocka F. E. (1974) *Appl. Microbiol. 27. 1131–1133.*

ENTEROBACTERIACEAE

ONPG DISCS

Code: DD13

For the rapid detection of beta-galactosidase activity in micro-organisms.

Contents
1 cartridge. Each contains 50 discs.

Disc Contents
Each disc is impregnated with phosphate buffered O-nitrophenyl-β-D-galacto-pyranoside (ONPG).

Description
Lactose fermentation is a classical identification test for many organisms. It is normally demonstrated by acid production after the disaccharide has been cleaved into galactose and glucose by the enzyme beta-galactosidase. However, it is essential for the lactose to be conveyed into the cell by a specific galactoside-permease enzyme before such cleavage.

The role of these two essential enzymes is important in the classification of micro-organisms into:

1 Active lactose fermenters (taking 18–24 hours) possessing both permease and galactosidase P+G+.
2 Delayed lactose fermenters (taking longer than 24 hours) lacking permease but possessing galactosidase: P– G+.
3 Non-lactose fermenters lacking both permease and galactosidase: P– G.

For the ONPG test[1] a synthetic galactoside (ortho-nitrophenyl-β-D-galacto-pyranose) is substituted for lactose. It is hydrolysed in the same way as lactose but the ortho-nitrophenol is chromogenic and when cleaved off in alkaline solution it produces a yellow solution:

Bacterium + ONPG (colourless) → hydrolysed β-galactosidase → O-nitrophenol (yellow)

This test is independent of an induced or constitutive permease enzyme and can be very rapid.

The ONPG test can be used to:

1 Differentiate late lactose fermenters (P– G+) from non-lactose fermenters (P– G–).
   (a) *Citrobacter (+)* and *Salm. arizonae* (+) from *Salmonella* (–).
   (b) *Escherichia coli* (+) from *Shigella sonnei* (–).
2 Aid in species differentiation.

(a) *Pseudomonas cepacia* (+) and *Ps. maltophila* (+) from other *Pseudomonas* species.

(b) *Neisseria lactamica* (+) from other *Neisseria* (–).

Technique
1 Place one disc into a sterile tube.
2 Add 0.1ml of sterile 0.88% sodium chloride (physiological saline).
3 Pick the colony under test with a sterile loop and emulsify it in the tube containing the disc and physiological saline.
4 Incubate at 35°C.
5 Examine at hourly intervals for up to 6 hours to detect active lactose fermenters.
6 Organisms that are negative after 6 hours should be incubated for up to 24 hours to detect the late lactose fermenters.

Interpretation of Results

Colourless	ONPG Negative
Yellow	ONPG Positive

The reaction speed depends on the size of inoculum.

Quality Control
Use known positive and negative beta-galactosidase producing organisms to monitor the disc reactions.

Reference
1 Lowe G. H. (1962) *J. Med. Lab. Technol. 19. 21–25.*

GARDNERELLA VAGINALIS

METRONIDAZOLE DIAGNOSTIC DISCS (50MG)

Code: DD8

SULPHONAMIDE DIAGNOSTIC DISCS (1000MG)

Code: DD11

An aid in the identification of Gardnerella vaginalis.

Contents
DD8. 1 cartridge. Each contains 50 discs.
DD11. 1 cartridge. Each contains 50 discs.

Disc Contents

DD8 Metronidazole	50μg
DD11 Sulphonamide	1000μg

Description
Smith and Dunkelberg[1] reported that metronidazole, previously thought to inhibit obligately anaerobic bacteria only was found *in vitro* to inhibit the growth of facultatively anaerobic strains of *Gardnerella vaginalis* when using the agar diffusion method.

Small pleomorphic Gram negative rods associated with 'non-specific' bacterial vaginitis were recognised by Gardner and Dukes[2] as the aetiologic agent. They named the organism *Haemophilus vaginalis*. However, in obvious conflict with the accepted definition of the genus *Haemophilus* it does not have a requirement for Haemin (X factor), Nicotinamide Adenine Dinucleotide (NAD or V factor) or any other co-enzyme-like growth factor. On the basis of microscopic morphology, Zinnemann and Turner[3] argued that it should be reclassified in the genus *Corynebacterium* and suggested the name *Corynebacterium vaginale*.

Taxonomic studies[4,5] have led to the naming of a new genus *Gardnerella* for inclusion of the organisms previously classified as *H. vaginalis* or *C. vaginale* with the type species *G. vaginalis*.

Bailey et al.[6] have recommended that the susceptibility and resistance to metronidazole and sulphonamide in conjunction with fermentation tests should be used as an aid in the separation of *G. vaginalis* from other possibly unrecognised biotypes of *G. vaginalis* or other vaginal bacteria that presumptively resemble the organism. They recommended a content of 50μg metronidazole per disc.

	Bacterial group or species *Susceptibility to disc of*	
	sulphonamide (1mg)	***metronidazole (50μg)***
G. vaginalis	R	S
Bifodobacteria and *G. vaginalis*-like organisms	S	S
Streptococci	R	R
Lactobacilli	R	R

S: Susceptible R: Resistant

It has been shown[7] that in the treatment of *G. vaginalis* – associated vaginitis with metronidazole, the hydroxy metabolite may contribute a significant antimicrobial effect, in view of its excellent activity *in vitro.* The diagnosis and treatment of non-specific vaginitis has been reviewed[8].

Technique
Inoculate the isolation medium with the specimen and place a 50μg metronidazole disc and a 1000μg sulphonamide disc on an area of the plate where heavy, but not confluent, growth can be expected. Smith and Dunkelberg[1] incubated at 35°C in air containing approximately 8% carbon dioxide, but more recently Ralph et al.[9] in a study of MICs of a number of antibiotics for *G. vaginalis* reported that susceptibility to metronidazole was significantly increased by incubation in an anaerobic atmosphere and more reliable results may be expected by incubation under these conditions.

G. vaginalis is best isolated on Columbia Agar CM331 with Gardnerella vaginalis Supplement SR119. See Section 2.

Quality Control
Use known strains of *G. vaginalis* to monitor the performance of the discs.

References
1 Smith R. F. and Dunkelberg W. E. (1977) *Sex. Trans. Dis. 4. 20–21.*
2 Gardner H. L. and Dukes C. D. (1955) *Am. J. Obstet. Gynecol. 60. 962–976.*
3 Zinnemann K. and Turner G. C. (1963) *J. Pathol. Bacteriol. 85. 213–219.*
4 Piot P., Van Dyke E., Goodfellow M. and Falkow S. (1980) *J. Gen. Microbiol. 119. 373–396.*

5 Greenwood J. R. and Pickett M. J. (1980) *Int. J. Syst. Becteriol. 30. 170–178.*
6 Bailey R. K., Voss J. L. and Smith R. F. (1979) *J. Clin. Microbiol. 9. 65–71.*
7 Ralph E. D. and Amatnieks Y. E. (1980) *Sex. Trans. Dis. 7. 157–160.*
8 Clay J. (1981) *J. Antimicrob. Chemotherapy 7. 501–504.*
9 Ralph E. D., Austin T. W., Pattison F. L. M. and Schieven B. C. (1979) *Sex. Trans. Dis. 6. 199–202.*

HAEMOPHILUS SPECIES

X FACTOR DISCS

Code: DD3

V FACTOR DISCS

Code: DD4

X + V FACTOR DISCS

Code: DD5

Discs impregnated with growth factors for the differentiation of the Haemophilus group of bacteria.

Contents
1 cartridge. Each contains 50 discs.

Description
Paper discs impregnated with growth factors for the differentiation of the *Haemophilus* group of bacteria. *Haemophilus* and *Bordetella* species may be identified according to whether basal media require the addition of 'X' and 'V' growth factors before growth will occur. X factor (haemin) and V factor (coenzyme I) may be directly incorporated into a basal medium or, more conveniently, are impregnated into paper discs or strips which are placed upon the surface of an inoculated basal medium. Colonies of those species which show growth only in the vicinity of a disc or strip impregnated with the particular growth factor are unable to synthesise it in optimal amounts[1].

Technique
Evenly inoculate the surface of a Blood Agar Base plate (*CM55 without blood)* with the organism to be tested and aseptically apply the Diagnostic Discs in the following positions around the periphery of the plate (approximately 1 or 2cm in from the edge of the medium):

X Factor Disc	12 o'clock
V Factor Disc	4 o'clock
X+V Factor Disc	8 o'clock

Incubate overnight at 35°C or for 48 hours if necessary, and observe for growth or no growth in the neighbourhood of a disc. If the organism requires X factor alone, it will grow only in the vicinities of the X and X + V factor discs; if it requires V factor alone, it will grow only in the vicinities of the V and the X + V factor discs; if both X and V factors are required, it will grow only in the vicinity of the X + V factor disc.

Growth of Bacterial Species with and without X and V factors

	No growth factors	*With X factor*	*With V factor*	*With X + V factor*
H. influenzae	–	–	–	+
H. aegyptius (Koch-Weeks bacillus)	–	–	–	+
H. parainfluenzae	–	–	+	+
H. ducreyi	–	+	–	+
*B. pertussis**	+	+	+	+

+Growth –No growth

*Requires special media for initial isolation, e.g. Bordet-Gengou medium, but laboratory strains show adaptation.

N.B. –V and X + V FACTOR DISCS MUST BE STORED AT –20°C to –10°C.

Quality Control
Use known strains of *Haemophilus* species to monitor the performance of the discs and the medium.

Reference
1 Kilian M. (1980) *Haemophilus. in Manual of Clinical Microbiology. Eds. Lennette et al. Amer. Soc. for Microbiol. 3rd Edn. Washington.*

STREPTOCOCCUS PNEUMONIAE

OPTOCHIN DISCS

Code: DD1

For the differentiation of Streptococcus pneumoniae.

Contents
1 cartridge. Each contains 50 discs.

Description
Bowers and Jeffries[1] have shown that there is complete correlation between bile-solubility and full 'Optochin' susceptibility for the differentiation of *Strept. pneumoniae* from other streptococci. Oxoid 'Optochin' Discs are paper discs ready impregnated with 'Optochin' (ethylhydrocuprein hydrochloride) which provide a convenient and reliable alternative to the bile-solubility test. Pneumococci are sensitive to 'Optochin' so that a culture shows a zone of inhibition around the impregnated disc, whilst streptococci either grow right up to the edge of the disc or, occasionally, show a very small zone of inhibition.

Technique
Streak a pure culture of the organism to be tested across one half of a Blood Agar plate (Blood Agar Base CM55 with 7% of sterile blood) and apply an 'Optochin' Disc immediately before incubation. At the same time apply a second 'Optochin' Disc to the other half of the plate, previously streaked with a known pneumococcus, in order to provide a positive control. After incubation, pneumococci show a zone of inhibition at least 5mm from the edge of the disc, whilst streptococci are completely resistant or show a small zone of inhibition extending not more than 2mm from the edge of the disc.

Oxoid 'Optochin' Discs may also be placed on the primary culture plate, before incubation, in order to provide rapid indication of the presence of large numbers of pneumococci.

Quality Control
See under Technique.

Reference
1 Bowers E. F. and Jeffries L. R. (1955) *J. Clin. Path. 8. 58.*

STREPTOCOCCUS PYOGENES (GROUP A)

BACITRACIN DISCS

Code: DD2

For the differentiation of Lancefield Group A from other beta-haemolytic streptococci.

Contents
1 cartridge. Each contains 50 discs.

Description
Sterile paper discs each containing 0.05 units of bacitracin, for the differentiation of Lancefield Group A from other beta-haemolytic streptococci. When used as a screening test prior to serological grouping, Bacitracin Discs effect considerable economies in time, labour and materials.

Maxted[1] showed that Group A streptococci were more sensitive to bacitracin than beta-haemolytic strains of other groups, and that bacitracin might therefore be used as a rapid diagnostic agent for Group A streptoccoci. He found that only 1.7% of 2,386 bacitracin sensitive strains proved not to be Group A, and only 2.5% of 851 resistant strains were Group A. Levinson and Frank[2] who employed impregnated paper discs for the same purpose, observed that 93.2% of 866 sensitive beta-haemolytic streptoccoci belonged to Group A. Streamer et al.[3] compared bacitracin disc, fluorescent antibody and Lancefield precipitin techniques for the identification of Group A streptococci. The bacitracin disc technique was considered to be the simplest and most practical for the routine clinical laboratory.

The use of bacitracin sensitivity testing is not restricted to the differentiation of beta-haemolytic streptococci. Guthof[4] found sensitivity to optochin, bacitracin, and furacin to be a useful test for distinguishing *Aerococcus viridans* and *Streptococcus milleri* from enterococci and mitis streptococci.

Technique
A. *Pure Cultures*

1 Evenly inoculate the surface of a Blood Agar plate (Blood Agar Base No.2 CM271 with 7% Sterile Oxalated Horse Blood SR49) with a pure culture of the beta-haemolytic streptocccus to be tested.
2 Aseptically place a Bacitracin Disc on the inoculated surface.
3 Incubate for 18 to 24 hours at 35°C.
4 Examine for the presence of a zone of inhibition around the Bacitracin Disc. A zone indicates that the streptococcus is presumptively Group A – if desired, further confirmation is obtained by serological grouping.

B. *Clinical Material*

1 Inoculate a Blood Agar plate with the throat swab or other material. Spread the inoculum so as to ensure that discrete colonies occur on some portion of the plate – so that the species in a mixed growth may be determined.
2 Aseptically place a Bacitracin Disc on the secondary area of inoculation.
3 Incubate for 18 to 24 hours at 35°C.
4 Examine for zones of inhibition. Organisms other than beta-haemolytic streptococci are sensitive to bacitracin, so that the presence of a zone of inhibition does not necessarily indicate Lancefield Group A streptococci. It is possible to select presumptive Group A streptococci if attention is given to colonial morphology. Further confirmation is obtained by serological grouping.

Bacitracin Discs DD2 are not recommended for routine testing of the sensitivity of organisms to Bacitracin. For this purpose it is advisable to employ Bacitracin Sensitivity Discs at a level of 10 units.

Quality Control
Use known Group A and non-Group A streptococci to monitor the accuracy of the discs and inoculum.

References
1 Maxted W. R. (1953) *J. Clin. Path. 6. 224–226.*
2 Levinson M. L. and Frank P. F. (1955) *J. Bact. 69. 284–287.*
3 Streamer C. W. et al. (1962) *Amer. J. Dis. Children 104. 157–160.*
4 Guthof O. (1960) *Ztschr. f. Hyg. u. Infektionskr. 146. 425–432.*

VIBRIO SPECIES

0129 DISCS

Codes: DD14 and DD15

For the differentiation of vibrios from other Gram-negative rods.

Contents
DD14 1 cartridge. Each contains 50 discs.
Disc content 0129 10μg per disc.

Description
The sensitivity of vibrios to the vibrio static agent 0129 (2,4-Diamino-6,7-di-iso-propylpteridine phosphate) has long been recognised[1]. This test is of great value in differentiating vibrios from other Gram-negative rods and particularly from aeromonads, which are characteristically resistant to 0129. The degree of sensitivity of vibrios to 0129 can also be used as a diagnostic feature in differentiation of *Vibrio* species (see Table 1).

Negative 0129 tests must be interpreted with caution. Strains of *V. cholerae* resistant to 0129 and trimethoprim have been reported[2]. Both plasmids and transposons appear to be responsible[3].

Specialised sensitivity testing media which contain low levels of sodium chloride should not be used as these may give poor growth of halophilic vibrios and may also give false positive results with Enterobacteriaceae[4]. Blood Agar Base No.2 CM271 can be recommended for such testing.

Table 1*
Sensitivity of Vibrio Species to 0129

	MIC (mg/ml)	*Disc Test*
Aeromonas species	400	Resistant
V. natriegens	40–60	Partially sensitive
V. alginolyticus	1–50	Partially sensitive
V. parahaemolyticus	15–40	Partially sensitive
Group F	7.5–50	Partially sensitive
V. harveyi	10–20	Partially sensitive
V. campbellii	3–20	Partially sensitive
Plesiomonas species	2–40	Sensitive
V. cholerae	2–7.5	Sensitive
Non-Cholera vibrios	2–7.5	Sensitive
V. metschnikovii	2–7.5	Sensitive
V. anguillarum	1–5	Sensitive
V. pelagia	1–5	Sensitive

Interpretation of Disc Test Results

150μg Disc	10μg Disc	
No zone	No zone	Resistant
Zone	No zone	Partially sensitive
Zone	Zone	Sensitive

* (Reproduced from 'The Vibrios' Public Health Laboratory Sciences 11, Monograph Series, page 21 with the permission of the Controller of Her Majesty's Stationery Office.)

Technique

1 Evenly incubate the surface of a blood agar plate made from Blood Agar Base No.2., CM271.
2 Place one DD14 and one DD15 disc on each plate.
3 Incubate at 35°C for 24 hours.
4 Observe for zones of inhibition.

Quality Control

Use known strains of 0129 sensitive and resistant organisms to monitor the performance of the discs.

References

1 Shewan J. M. and Hodgkiss W. (1954) *Nature 63. 208–209.*
2 *The Lancet*, August 8th 1992, *340. 366–367.*
3 Gerbaud G., Dodin A., Goldstein F. and Courvain P. (1985) *Ann. Inst. Past./Microbiol. 136B. 265–273.*
4 Furniss A. L., Lee J.V. and Donovan T.J. (1978) *'The Vibrios'. Public Health Laboratory Service, Monograph Series. Number 11.*

BLOOD PRODUCTS

Horse blood, defibrinated	SR50
Horse blood, oxalated	SR49
Horse blood, haemolysed	SR48
Sheep blood, defibrinated	SR51
Horse serum	SR35

Horse and sheep blood are the most widely used animal blood products in culture media. The choice of animal is largely traditional, with the USA and much of continental Europe preferring sheep blood, whilst the UK and Commonwealth partners prefer horse blood.

The haemolytic reactions of horse and sheep blood are not identical and blood agar media designed for horse blood may not be satisfactory with sheep blood and vice versa. See Blood Agar Base (Sheep) CM854 Section 2.

It is not possible to sterilise whole blood products and therefore they must be collected aseptically. Freshly drawn blood from healthy animals can destroy small numbers of bacteria which may transiently infect it during collection but considerable expertise and skill are required to maintain high quality, sterile blood. It is difficult to obtain sterile blood from abattoirs therefore bleeding is carried out in suitable premises from animals which are specially maintained on high protein diets.

Defibrination is now accepted as the best method of preventing blood clotting. It must be carried out immediately after the drawing the blood and the agitation must be sufficient to denature the fibrinogen but not to cause rupture of the erythrocytes and haemolysis. Some lysis is inevitable in this process but the release of NAD in horse blood from the ruptured erythrocytes stimulates the growth of *Haemophilus influenzae*. This effect is not seen with sheep blood.

The use of potassium oxalate as an anticoagulant increases the yield of whole blood but it remains as a potential chelate of divalent metals. This chelate effect, which also applies to sodium citrate-treated blood, can be seen in the overall smaller colonies which grow on anticoagulant-containing blood agar.

To prolong the shelf life of blood products in the user's hands, blood is collected, bottled and dispatched on the same day. The physical characteristics of appearance, haemoglobin content and packed cell volume are checked before release but sterility tests are carried out beyond the time of dispatch. Should samples of individual animal blood collections show infection then all recipients of the suspect blood are notified by telephone, fax or telex.

Haemolysed or laked horse blood is used for special purposes in culture media. It has been used for many years in *Corynebacterium diphtheriae* media, where better growth was observed after lysis of the horse blood by the tellurite in the medium.

Lysed blood is stimulatory for many *Clostridia* and *Haemophilus* species but Fildes Peptic Digest of Blood SR46 can also be used for this purpose.

In antibiotic susceptibility testing, lysed horse blood is added to the medium to improve the reactions with trimethoprim and sulphonamides. Most culture media, unless specially processed for susceptibility testing, contain amounts of thymidine which can antagonise the inhibitory effects of these antimicrobials. When horse blood is lysed the erythrocytes release an enzyme thymidine phosphorylase which converts thymidine into the much less antagonistic compound thymine. This enzyme does not exist in sheep erythrocytes. The lytic agent used for lysed horse blood is white saponin which appears not to affect the growth of bacteria at the level required for lysis.

Serum for Culture Media

Horse serum	SR35

Totally aseptic processing is not required for blood serum production. Blood is collected in clean vessels in a clean environment. After clotting has taken place the blood is stored at 2–8°C to allow the clot to retract

and the serum separate. The separated serum is then filter-sterilised and filled out into sterile bottles. All sera should be stored at 2–8°C but for longer term storage it is best frozen at –20°C.

Serum can become cloudy on storage caused by lipase enzymes and lipid changes. Heating the serum to 56°C for 30 minutes (inactivation) will usually overcome this problem.

Sheep Erythrocytes for Immunological Reactions

Sheep blood (in formaldehyde)	SR52
Sheep blood (in Alsever solution)	SR53

After washing the sheep erythrocytes, by centrifuging with physiological saline until the supernatant saline is free from haemolysis, the washed cells may be used for any immunological/serological test where sheep cell antigens are required e.g. Rose-Waaler, Paul Bunnell and various virological complement fixation tests.

The sheep blood should be stored at 2–8°C and used before the expiry date on the label.

6
ANTIMICROBIAL SUSCEPTIBILITY TESTING

November 1998

ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST)

The Oxoid system of AST is the disc diffusion method which although developed in 1947[1] remains as the most widely used test. Performed with care, using adequate controls, it is as accurate as more costly and complicated tests.

In common with all other methods it cannot mimic the *in vivo* environment but it does uniquely show the effect of a changing antimicrobial concentration (first rising and then falling) on an increasing microbial population. The minimum inhibitory concentration (MIC) of the antimicrobial appears at the edge of the zone of inhibition which represents the interaction of a critical concentration of antimicrobial at a critical time on a critical microbial population[2].

In October 1997 Oxoid *aura* was launched. This enhances the AST system by using an accurate and objective electronic zone size measurement facility linked to unique species specific interpretative software.

Oxoid *aura* ensures high reproducibility and better separation of Susceptible, Intermediate, Resistant (S,I,R) categories with the facility to store and process data for future comparison and epidemiological studies. (See page 6-3.)

The methods are simple but the above interactions are very complex and the accuracy and precision of the test is based on the following critical components:

1 CULTURE MEDIUM

The medium chosen for the test must have been manufactured and tested specifically for AST. Oxoid manufactures and recommends the following agar media:

i Diagnostic Sensitivity Test Agar (DST Agar) CM261 which was the first medium specifically designed for this test. It is still very popular but it has been modified to reduce its thymidine content and it is therefore less useful as a diagnostic medium.

ii HR Medium CM845 – a chemically defined medium for susceptibility testing with antifungal agents.

iii HTM (Haemophilus Test Medium) CM898 was specifically formulated for the susceptibility testing of *Haemophilus influenzae*. The medium is based on Mueller-Hinton Agar to which is added a supplement (SR158). The supplement contains NAD and Haematin.

iv Iso-Sensitest Agar CM471 was developed as a semi-synthetic AST medium in which undefined protein hydrolysates were reduced to the minimum level which would allow optimum growth of a wide range of organisms and the 'free' cation strength adjusted to give correct MIC results. Its popularity rests on its reproducible performance.

v Mueller-Hinton Agar CM337 was originally formulated to grow pathogenic neisseria and it was adopted for use in the Bauer-Kirby test[4]. Its variable performance was strongly criticised but following agreement between culture media manufacturers and representatives of NCCLS and FDA a uniform medium is now produced which must meet the NCCLS specifications[5]. Therefore Mueller-Hinton medium suitable for AST will carry a statement on the label that it meets NCCLS standards.

vi Sensitest Agar CM409 the first semi-defined AST medium which remains popular with laboratories which have designed methods around this specific formulation[3].

vii Wilkins-Chalgren Anaerobic Agar CM619 was developed as a medium for the growth of anaerobic bacteria. Its relative lack of inhibitory action towards anaerobes made it the medium of choice for anaerobic agar dilution tests[6].

Anaerobic disc diffusion tests can be carried out but it is essential to include adequate controls which can monitor the effect of anaerobiosis on antimicrobials.

All the above media have broth versions of their formulations (except DST and H.T.M.). This allows dilution or pre-enrichment to be carried out in the same medium. It is also helpful when carrying out MIC tests in broth to standardise disc diffusion zones to establish the breakpoint categories of S,I,R.

Many of the constituents used in culture media affect the precision and accuracy of AST results. The agar must allow free diffusion of the antimicrobial from the disc. Variations in pH and ionic strength will cause differences in zone sizes. Blood can reduce the zone size of highly protein-bound antimicrobials e.g. fusidic acid. Changes in 'free' electrolyte content will affect aminoglycosides, tetracyclines and polymyxins. Glucose will enlarge the zones of antimicrobials against organisms which are adversely affected by a fall in pH following fermentation of the sugar. Thymidine and thymine levels have to be monitored and reduced, if necessary, to prevent antagonism of trimethoprim and sulphonamides. All AST media must be specifically tested with critical 'drug-bug' combinations to measure their performance and to ensure that they meet the quality specifications. The latter tests are carried out by the manufacturer, especially to ensure that the medium conforms to regulatory standards, they should also be carried out in an abbreviated form in the user laboratory to monitor the performance of all of the components of AST.

2 ANTIMICROBIAL DISCS

The paper discs used in the diffusion method are made from paper which conforms to the WHO[7] and FDA[8] standards.

Impregnation of the discs ensures that accurately prepared solutions of antimicrobials are uniformly spread across the paper. The drying procedures used do not affect this uniform distribution or cause deterioration in activity of the antimicrobial.

When each cartridge of 50 discs is sealed together with a silica gel capsule in a foil-covered see-through blister, the discs contain less than 2% moisture and

are stored at low temperatures. Shelf-life studies of the discs in their packaging demonstrate that they meet the stated storage life printed on the labels.

Finally, samples are taken from every batch/lot manufactured and the discs tested microbiologically to confirm that the antimicrobial content lies within 80–120% of the stated content on the label.

Storage
Discs must be stored at –20°C if kept for long periods. Storage at 2–8°C is suitable for discs currently being used or to be used very soon. Discs should be returned to the refrigerator as quickly as possible after use. The most common cause of moisture reaching the discs and causing destruction of labile antimicrobials is condensation of warm laboratory air on cold discs removed from the refrigerator.

It is important to allow the cartridge blister pack to reach room temperature before exposing the discs, a period of one hour is generally sufficient. Use discs in order of expiry date, which is valid only for unopened blisters stored under proper conditions.

3 INOCULUM

One of the very critical factors for accuracy and precision in disc diffusion tests is the inoculum preparation. It is therefore important to use a technique which will always yield a uniform suspension of the correct number of organisms, 10^5–10^6 orgs/ml.

Various techniques are described in which suspensions of pre-grown organisms are prepared or small inocula are incubated for fixed periods of time. It is important that more than one colony is sampled (4–10 cols) to ensure a representative sample of the organism has been taken.

Some form of standardisation of the final suspension is necessary and it should be noted that different organisms will display different opacities of solution to yield a dense but not confluent growth.

To complement the newly launched Oxoid *aura* System (see page 6-3) Oxoid have launched the Oxoid Turbidometer. This instrument provides the inoculum density standardisation necessary to ensure accurate reproducible results.

4 OTHER FACTORS INFLUENCING THE RESULTS

1 **Temperature of incubation** – the incubators should be checked for satisfactory performance and their recording thermometers should show air temperatures of 35–37°C with fluctuations of not more than 2°C. Agar plates should not be placed in high stacks because the middle plates will take longer to reach the incubator temperature and this delay could cause overlarge zones.

2 **Pre-incubation and pre-diffusion conditions** – a routine procedure should be established so that inoculated plates have discs applied not later than 15 minutes after inoculation. This prevents a pre-incubation of organisms before the antimicrobial discs are applied. Similarly once the discs have been applied, plates should be placed in the incubator within a 15 minute interval, to prevent pre-diffusion of the antimicrobial at room temperature.

3 **Uniformity of agar depth** – plates should be poured on levelled surfaces, using dishes with flat bottoms, to ensure a uniform depth of agar (3–4mm in depth).

4 **Application of discs** – it is essential that discs are in intimate contact with a moist agar surface. Therefore either use dispensers which have a tamping action on the discs or press them separately after application. Do not overdry the agar surface before applying the discs. Do not attempt to place too many discs on the agar surface; six 6mm discs are ideal for a 90mm dish. Some workers apply up to 12 discs onto a 150mm dish.

5 **Incubation period** – ensure uniform times of incubation for the plates, 16–18 hours at 35–37°C is usually satisfactory.

6 **Interpretation of zone sizes** – after incubation the plates are removed and the zones of inhibition noted and measured. (See Oxoid *aura* page 6-3.)

The diameter of the zone (including the diameter of the disc) is measured to the nearest millimetre, using calipers. If the NCCLS Bauer-Kirby method is being used then the zone diameters can be compared with the current NCCLS Standards which can be obtained from the National Committee for Clinical Laboratory Standards, NCCLS Publications, Villanova, Pa. USA. This standard should not be used unless the test is carried out following the described methodology on Mueller-Hinton medium with the appropriate discs.

Where other systems are used the zone size breakpoints should have been determined using MIC/zone comparative tests, following the particular methodology chosen.

7 **Control cultures** – it is essential that each laboratory maintains adequate control over AST methods by testing reference cultures at regular intervals. Daily tests may be required if new media and discs are constantly being used.

The following reference strains are used in the NCCLS test method.

Staphylococcus aureus ATCC® 29213

Enterococcus faecalis ATCC® 29212

Escherichia coli ATCC® 25922

Pseudomonas aeruginosa ATCC® 27853

Escherichia coli ATCC® 35218 for testing clavulanic acid compounds.

Records of the results obtained with these reference strains should be maintained in log books and any deviation of zone sizes from the range accepted should be investigated.

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Note
The list of antimicrobial discs manufactured, the agents, generic and proprietary names, the symbols and range of levels available are supplied in the Oxoid Product List and the list is updated annually.

Antimicrobial Susceptibility Discs

Discs in routine use should be stored at 2 to 8°C. Longer term storage should be at –20°C. After cold storage allow discs to reach room temperature before opening storage containers. Discs are presented in cartridges for dispensing either individually with Oxoid ejectors or with the Oxoid Dispenser System. There are 50 discs per cartridge, 5 cartridges per pack.

Cartridge packs
Each cartridge is individually sealed together with a silica gel capsule in a foil-covered see-through blister. Designed to allow the microbiologist better control and storage of the discs in use.

THE OXOID DISC DISPENSER MKIII

An enhanced system for antimicrobial susceptibility testing incorporating the new ergonomically designed Disc Dispenser. It has a simple one-handed operation and is fully height-adjustable to cater for various depths of media. The cover and base of the dispenser are fully interlocked to prevent the ingress of moisture: cartridges 'click' positively into their correct locations and a plastic skirt ensures that each agar plate is precisely centred every time the dispenser is used.

Product	*Pack size*	*Order Code*
Disc Dispenser Mk III (90mm) for 90mm petri dishes		
Disc Dispenser Mk III (90mm) for 6 cartridges	1	ST6090
Disc Dispenser Mk III (90mm) for 8 cartridges	1	ST8090
Disc Dispenser Mk III (100mm) for 100mm petri dishes		
Disc Dispenser Mk III (100mm) for 6 cartridges	1	ST6100
Disc Dispenser Mk III (100mm) for 8 cartridges	1	ST8100
Disc Dispenser (150mm) for 12 cartridges	1	ST1215
Ejector *for using cartridges individually*	6	HP053A
Replacement Dessicant Pack	1 pack	ST9100

Antimicrobial Susceptibility Testing Media

Media for Antimicrobial Susceptibility Testing (coded CM) should be stored in their closed containers at an even temperature in a cool, dry place, away from direct light.

Product	*Pack Size*	*Order Code*
Diagnostic SensitivityTest Agar Base (DST Agar)	500g	CM261B
HR Medium	100g	CM845A
Haemophilus Test Medium (HTM)		
HTM Supplement	10 vials	SR158E
HTM Base	500g	CM898B
'Iso-Sensitest' Agar	500g	CM471B
'lso-Sensitest' Broth	500g	CM473B
Mueller-Hinton Agar	500g	CM337B
Mueller-Hinton Broth	500g	CM405B
'Sensitest' Agar	500g	CM409B
Wilkins-Chalgren Anaerobe Agar	500g	CM619B
Wilkins-Chalgren Anaerobe Broth	500g	CM643B

OXOID *aura*

The importance of standardisation.

Antimicrobial Susceptibility Testing by the disc diffusion method is simple, flexible, informative and cost-effective[1].

However, a recent survey demonstrated that, between laboratories, there is ''considerable variation in the medium used, the method of inoculum preparation and application, disc concentrations, incubation conditions and the basis of interpretation of zone sizes''[2].

It is generally accepted that there is a need for greater standardisation of methodology and interpretation of results to ensure accurate, reproducible results, within and between laboratories.

The Oxoid *aura* database enables clinical isolates to be compared to an appropriate standard for every antibiotic and concentration in the system. The comparison is performed at species level and automatically generates interpretation of the result.

Interpretations are based on the principles set forth by the Swedish Reference Group of Antibiotics (SRGA) and its sub-committee on Methodology (SRGA-M)[3]. The value and function of this system has been proven over many years.

The Complete Antimicrobial Susceptibility Testing (AST) System.

Accurate, reproducible AST results depend on the following:

- High quality products used in a defined methodology
- Standardised inoculum density
- Precise zone size measurement (*aura*)
- Accurate and meaningful interpretation of results (*aura*)

All of this is achievable using the complete Oxoid AST System:

Components of the System:

Specialist culture media	Range includes Mueller-Hinton Agar and Iso-Sensitest Agar.
Antimicrobial Susceptibility Discs	See Product List for full range
Disc Dispensers	6, 8 and 12 Place
Turbidometer	Standardised inoculum density
Interpretative database	Performed at species level – generates interpretation of the result

References

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2 Andrews J. M. et al (1996) *Antimicrob. Chemother. 37: 187–204.*

3 Report from the Swedish Reference Group of Antibiotics and its Methodology Subcommittee (SRGA-M), 1991.

In addition to Culture Media, Susceptibility Discs, Dispensers and the Oxoid Turbidometer the full Oxoid *aura* system is as follows:

Product Description	*Product Code*
aura Callipers	AU101A
aura Barcode Labels (1–50,000)	AU102A
aura Barcode Labels (50,000–100,000)	AU103A
aura Barcode Reader	AU104A
aura System Software	AU106A
Oxoid Turbidometer (UK)	AU107A
Oxoid Turbidometer (International)	AU108A

Separate literature available.

7
ANAEROBIC SYSTEMS

November 1998

ANAEROBIC BACTERIOLOGY

THE ROLE OF OXYGEN

The fact that life almost certainly evolved in an environment which lacked oxygen was not originally appreciated. At the time of Pasteur there was no precedent that life could exist in the absence of oxygen. When, in 1861, Pasteur declared that some organisms could exist without oxygen and appeared to die in its presence, the theory of the toxicity of oxygen was born.

The beginning of this theory was Pasteur's observation, looking down a microscope, that rapidly moving bacteria in a fermenting sugar solution ceased moving at the periphery of the drop, whilst remaining actively motile in the centre. Such organisms he called 'anaerobies' as opposed to 'aerobies' who depended on oxygen for their survival.

ANAEROBIC BACTERIA

Pasteur's fermentation studies on yeasts showed that these organisms could multiply with or without air and changed their metabolism of sugar to suit the gaseous environment. Thus facultative anaerobes, which could adapt and survive, were distinguished from strict anaerobes and from strict aerobes[2]. In 1893 Veillon[3] isolated a micrococcus which was strictly anaerobic (obligate anaerobe). Later, in 1897, Veillon and Zuber[4] described various pathological conditions where anaerobic bacteria could be the cause of the infective condition.

Further work, between 1898–1902, linked anaerobes to several human pathologies including empyaema, female genital infections, gangrene of the lung etc.

The anaerobic bacteriology of botulism, gas gangrene and tetanus soon became familiar to clinicians because the causative organisms, members of the *Clostridium* genus, were relatively easy to cultivate. Other anaerobes remained obscure, even though they were responsible for common supperative conditions. The reason for their obscurity was the cumbersome and difficult techniques required for their cultivation.

Anaerobic methods in liquid or semi-solid media
Methods to cultivate anaerobic organisms in liquid media were quickly developed because simple boiling expelled most of the dissolved oxygen. The addition of a heated iron strip or meat granules to the broth maintained the low oxygen levels.

A small amount of agar (0.2% w/v) in the broth was also effective by increasing the viscosity of the liquid and slowing down the re-absorption of air in the cooled medium.

THEORY OF OXYGEN TOXICITY

The addition to media of Eh reducing agents, such as thioglycollate or cysteine which contain sulphydral groups, is protective to anaerobic organisms under certain circumstances. The oxidation-reduction potential (Eh) of an environment or medium is measured in mV and expressed as the tendency either to accept electrons (become reduced) or donate electrons (become oxidised). The Eh of normal tissue is +150mV but in the colon it can be as low as –250mV.

The question of limiting levels of Eh which allow anaerobes to grow has not been determined because of the relative influence of oxygen and Eh. It appears that the presence of oxygen is of greater importance because it has been shown that the growth of anaerobes was inhibited in the presence of air, even though the Eh was –50mV. In the absence of air, anaerobic organisms grew in spite of positive Eh values[6].

The toxicity of oxygen occurs because of the radicals formed by electron capture along reduction pathways: superoxide O_2^-, singlet oxygen O_2^2 as well as hydroxyls and peroxides. The enzymes catalase, peroxidase and superoxide dismutase are protective systems against these toxic radicals[7]. Aerobic organisms and facultative anaerobes have efficient systems of enzymes which can neutralise such toxic radicals; obligate anaerobes lack such protective systems.

Furthermore the addition of protective substances to culture media can overcome oxygen toxicity produced biologically by the organisms or chemically in the medium and help protect vunerable organisms. Examples of such substances are blood, charcoal, iron salts, pyruvate, cysteine.

Anaerobic methods for solid media
Anaerobic bacteria can be grown in solid agar media by making deep stabs in tubes of agar or adding a suspension of the organism to molten, cooled agar before pouring butts or plates of agar media. The latter method usually requires an agar overlay to ensure anaerobic growth.

However, submerged colonies of bacteria are difficult to access and investigate further. It would not have been possible to make good progress in anaerobic bacteriology without the ability to produce separate colonies of organisms on the surface of agar plates. To achieve this goal, systems of anaerobic cultivation for agar plates had to be created.

Metal or glass jars with gas-tight lids that had valves fitted to them were developed. Air could be evacuated from the jar through a valve and oxygen-free gas flushed through the jar. A more efficient method came with the use of a palladium catalyst fixed to the underside of the lid and hydrogen gas added to the jar. The necessity to heat the catalyst and the potentially violent combination of oxygen and hydrogen to form water, gave an ever present risk of explosions.

It was the development of the 'cold' catalyst, in a flameproof capsule, and the replacement of glass with polycarbonate plastic which made the process much safer. A further development was the replacement of gas cylinders by foil sachets of chemicals which, when activated with water, produced hydrogen in sufficient quantities to reduce the oxygen level in the jars below 1% v/v. These sachets also supply carbon dioxide at a level of 5–8% in the jar to improve the growth of many anaerobes. Further modifications of the chemicals used in the sachets enabled atmospheres to

be created which would support micro-aerophilic organisms (about 6% oxygen in the jar) or capnoeic organisms (about 10% CO_2).

Anaerobic indicators will show whether the redox potential (Eh) in the jar has been reduced below –50mV, which is the level required to change the resazurin indicator from pink to white.

Collection and transport of anaerobic specimens
It must be emphasised that in spite of every care taken in cultivation, poorly collected and transported samples will yield poor or negative results.

Samples for anaerobic investigation should be collected with care and protected from air. Swabs will not be suitable, unless they are specially treated[8] or placed in Stuarts Transport Medium[9]. Generous samples of pus or fluids do not need anaerobic transport but they should not be unduly delayed before examination[10].

It was Dack who said 50 years ago that it should be possible to isolate and study anaerobes in a relatively convenient, routine fashion using 'modern apparatus'[11]. Much later and armed with the components described below, anaerobic bacteriology is within reach of all microbiological laboratories.

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ANAEROBIC SYSTEMS

For some time now Oxoid has been marketing two distinctly different systems used for growing bacteria which require special atmospheres including anaerobic organisms, microaerophilic organisms and CO_2 dependent organisms.

The original Oxoid system consisted of an anaerobic jar of advanced design (Code HP11) together with a gas generating kit for anaerobes (BR38), a CO_2 gas generating kit (BR39) and gas generating kits for Campylobacter (BR56 and BR60).

In the case of BR38, BR56 and BR60 water is added to the sachets to initiate a reaction and the production of hydrogen and CO_2. A low temperature catalyst (BR42) is required to be used in the anaerobic jar on each occasion.

THE OXOID ANAEROBIC JAR HP11

A 3.4 litre capacity Anaerobic Jar of advanced design that gives great flexibility in use by coping equally well with Gas Generating Envelopes or Gas Cylinders.

Both Jar and Lid are of robust construction and used with the Low Temperature Catalyst BR42 ensure unprecedented protection to operator and equipment.

The Oxoid Anaerobic Jar has a number of novel design features and for greater convenience in use it is supplied with a corrosion-resistant plate carrier that greatly reduces the time and effort needed to load the jar. A test tube carrier is available as an optional extra minimising the risk of spillage of broth cultures.

Anaerobiosis is achieved rapidly, safely and efficiently using the Gas Generating Kit BR38 or hydrogen obtained from cylinders.

The Oxoid Anaerobic Jar may also be used for the isolation of microaerophilic and CO_2-dependent organisms by using the Campylobacter Gas Generating Kit BR56 or the CO_2 Gas Generating Kit BR39.

Catalytic activity may be checked both by the pressure gauge for an immediate indication of efficiency and by the Anaerobic Indicator BR55 which provides supporting evidence of the pressure changes. These checks ensure that the absence of growth does not reflect poor anaerobic incubations.

The Oxoid Anaerobic Jar HP11 is part of the complete Oxoid Anaerobic System consisting of:

- 3.4 Litre Anaerobic Jar of advanced design.
- Gas Generating Kit which is superior in design to any other on the market.
- A new safe low temperature catalyst.
- An Anaerobic Indicator that is reliable and reacts faster than any other equivalent products.

OXOID GAS GENERATING KIT BR38

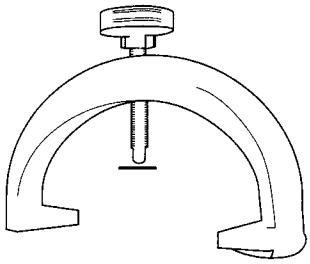
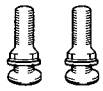
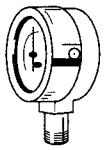
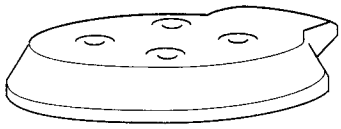
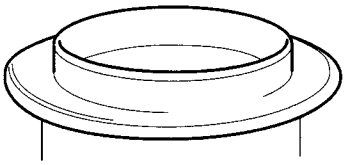
The Oxoid Gas Generating Kit BR38 is a laminated foil envelope presented with an inner container holding tablets of sodium borohydride, sodium bicarbonate and tartaric acid. The addition of water to the envelope activates the system causing hydrogen and carbon dioxide to be produced.

Gas production takes place smoothly and reproducibly because the porous membrane of the inner container regulates the passage of water inwards and gas outwards.

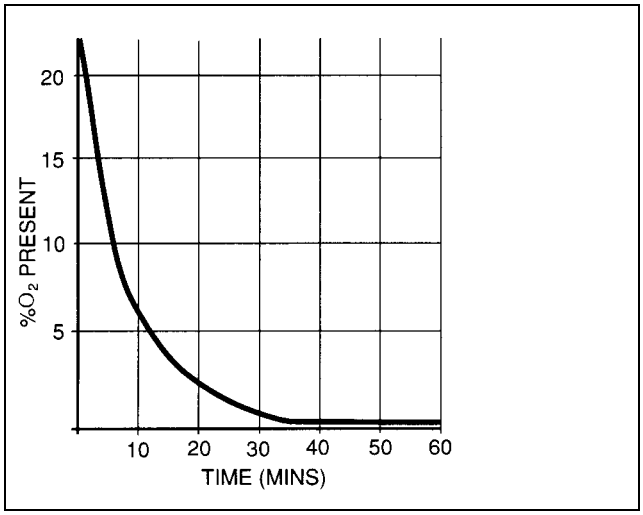
Each individual Gas Generating Kit when activated with water evolves sufficient hydrogen for the catalytic removal of oxygen present in the jar and leaves the final internal pressure approximating to that of the atmosphere.

Carbon dioxide is also evolved to give a final concentration of 10% v/v in the atmosphere.

Gas generation is completed within 30 minutes and because the resultant solution is acid it does not reabsorb the carbon dioxide so necessary for the growth of fastidious anaerobes.

Outstanding Design Features of the Oxoid Anaerobic Jar HP11		**How You Benefit**
Strong metal clamp		It won't break or distort. Large diameter tightening screw can be turned easily even by small hands.
Pressure release valve		Extra safety - controlled release of any excessive pressure build-up occurs at preset level. As valve re-closes when pressure is safe, you don't lose anaerobiosis.
Schrader® valves		Maximum versatility – use jar with either gas generating kits or bottled gas. Valve servicing is easy.
Easy-to-read pressure gauge		Quickly gives clear indication of poor catalyst performance. As you don't need secondary connections to measure vacuum, it saves you time and effort.
Attractively coloured rigid metal lid		Lid won't crack or distort. Its distinctive smooth finish is corrosion resistant and easily cleaned.
A really gas-tight seal		You can be confident no leakage will occur. O-ring can be simply replaced when needed.
Effective and safe catalyst		Sachet is specially designed to combine rapid efficient action with great safety. Maximum temperature reached is below flash point of hydrogen – about half that of conventional catalysts.
Heavy duty transparent jar		Strong, safe and you can see what's happening inside.

Rate of Oxygen catalysis
This graph shows the rapidity with which the oxygen level in the gas jar is lowered using the Oxoid Gas Generating Kit BR38.



GAS GENERATING KIT CARBON DIOXIDE SYSTEM BR39

Description
The Oxoid Carbon-Dioxide Generating Kit is a reliable and convenient method for producing suitable conditions, in standard jars, for organisms requiring an enhanced CO_2 atmosphere.

Each sachet contains two tablets, both of which are composed of tartaric acid and sodium bicarbonate. When used as directed, they will together produce 350ml carbon dioxide, which in the Oxoid Anaerobic Jar will give a final carbon dioxide level of approximately 10% (v/v) within 1½ hours.

The requirement of CO_2, by gonococci is well documented[1,2] although strains vary widely in their requirement for this gas.

Chapin[3] introduced the candle-jar producing approximately 2.5% (v/v) CO_2, but this is below the optimum level for the growth of carbon dioxide requiring gonococci, particularly if the number of bacteria is small[4].

The Carbon-Dioxide Gas Generating Kit may also be used to provide the enhanced CO_2 atmosphere required for growth of meningococci.

A 10% (v/v) CO_2 atmosphere is required for isolation of *Brucella* species[5,6]. Plates of Blood Agar Base No.2, CM271, or Brucella Medium Base, CM169, supplemented with 5–10% (v/v) inactivated horse serum and Brucella Selective Supplement, SR83, should be incubated at 35°C in a carbon dioxide enriched atmosphere for ten days and examined every two days.

Hale[7] recorded the isolation from an abscess of a dwarf colony *Staphylococcus aureus* which was dependent on CO_2 for characteristic growth.

Thomas and Cowlard[8] reported strains of *Staph. aureus* that grew normally in 1–2% of CO_2 but which on aerobic culture plates grew as minute unpigmented colonies that were coagulase and catalase negative.

Other reports[9,10,11] also concern dwarf variants of differing phage types of *Staph. aureus* that grow normally in a CO_2 enriched atmosphere.

Barker et al[12] have identified strains of *Klebsiella* species that are CO_2 dependent and Eykyn and Phillips[13] reported the isolation of a CO_2 dependent *Escherichia coli* from a urine specimen.

It can be important clinically that CO_2 dependent strains of such commonly occurring organisms are recognised and routine incubation of all specimens in CO_2 is recommended.

Disposal
After opening the jar, the exhausted sachet should be removed without spilling the contents. The solution remaining in the sachet is mildly acidic and may be poured away into a sink or flushed with running water. The empty sachet can then be discarded with normal laboratory litter.

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GAS GENERATING KITS FOR CAMPYLOBACTER BR56 & BR60

Description
The Oxoid Gas Generating Kits for Campylobacter isolation, BR56 and BR60 constitute a reliable and convenient method for producing suitable gaseous conditions, in standard jars, for organisms such as *Campylobacter* species which require a reduced oxygen atmosphere.

BR56 and BR60 are disposable gas generating kits that produce hydrogen and carbon dioxide in sufficient quantity that after reaction with a palladium catalyst in an anaerobic jar will produce an optimal gaseous atmosphere for the growth of campylobacters and other microaerophilic organisms. BR56 is designed for 3.0–3.5 litre jars and is suitable for the Oxoid Anaerobic Jar HP11 and for many other jars currently in use in laboratories. When used as directed, each sachet will produce about 1,000ml hydrogen and 350ml carbon dioxide.

BR60 is designed for 2.5–3.0 litre jars and is for use

with the small lightweight plastic anaerobic jar. When used as directed, each sachet will produce about 700ml hydrogen and 250ml carbon dioxide.

In each case a residual concentration of about 6% oxygen and 10% carbon dioxide in the jar is obtained in under 30 minutes.

Some variation in the oxygen level in each jar will occur, depending on the number of inoculated plates.

Use of an Anaerobic Indicator to check the efficiency of catalysis is inappropriate but an active catalyst will produce pressure changes which can be observed from the gauge fitted to the Oxoid Anaerobic Jar. After an initial pressure increase of approximately 0.1 bar, catalytic activity will lead to a pressure reduction to zero or even – 0.05 bar. Should the catalyst be inactive the pressure will rise to approximately 0.2 bar and be maintained.

Campylobacter species require some oxygen yet are inhibited by the amount in air. The preferred level of oxygen required for growth has been reported to be between 5 and 7%. This exacting level, together with a carbon dioxide requirement, has made isolation of these organisms from human and animal sources a complicated procedure[1].

Alternative methods for achieving the special atmosphere may be hazardous and give a wide variation in the residual oxygen level in the jar leading to erratic recovery of *Campylobacter* species.

The Gas Generating Kit for Campylobacter provides a reproducible atmosphere containing approximately 6% of oxygen and 10% of carbon dioxide.

An evaluation which compared different methods for producing microaerobic atmospheres confirmed the effectiveness of gas generating envelopes[2].

By using media made selective by the addition of Campylobacter Selective Supplements maximum recovery of the majority of *Campylobacter* strains will be achieved.

Directions

1 Cut off the corner of a sachet as indicated by the broken line. Avoid folding or crushing the sachet.
2 Add a measured 10ml of water.
3 Immediately stand the sachet upright in the anaerobic jar *fitted with an active catalyst* and close the lid according to the manufacturer's instructions. (If preferred, water may be added to sachets already located in the jar.) The use of the Oxoid catalyst is recommended, because of its high efficiency, combined with inbuilt safety features. Activity of the catalyst may be prolonged by ensuring that it is dry on each occasion it is used. Heating to 160°C for 90 minutes after each use is recommended.
4 Disposal. After opening the jar, the exhausted sachet should be removed without spilling the contents. The solution left in the sachet is mildly acid and may be poured away into a sink and flushed with running water. The empty sachet can then be discarded with normal laboratory litter.

Warning

Gas Generating Kits activated but not in gas jars should be kept away from unguarded flames and sparks. Once the reaction has subsided (after about 30 minutes) the sachet can be discarded as above.

Reference

1 Butzler I. P. and Skirrow M. (1979) *Clinics in Gastroenterology 8. 737–765.*
2 Bolton F. J., Wareing D. R. A. and Sails A. D. (1997) *Eur. J. Clin. Microbiol. Inf. Dis. 16. 839–842.*

THE OXOID ANAEROBIC CATALYST BR42

The Oxoid low temperature Catalyst BR42 is of patented design[1] and is a safer and more efficient version of the cold catalytic devices which are used universally to create low oxygen atmospheres within anaerobic gas jars.

Each Oxoid Catalyst contains 4 grams of palladium-coated pellets and is suitable for use in anaerobic jars up to 3.5 litres effective volume. This follows the recommendation of the United Kingdom Department of Health and Social Security[2] that there should be not less than 1 gram of catalyst for each litre volume of anaerobic jar. The extra large charge of activated palladium in the Oxoid Catalyst is wrapped in a special foil and enclosed in a large surface area capsule woven from very fine stainless steel wire.

The graph (right) shows the difference in operating temperature between a conventional catalyst (unprotected pellets enclosed in wire gauze) and the Oxoid Catalyst. The description 'safe' applied to the Oxoid Catalyst refers to the reduction in risk of an explosion in the Oxoid Anaerobic Jar. Explosions in Anaerobic Systems although rare, can occur when hydrogen and oxygen are present in critical proportions. Such explosions are triggered by extremely hot catalyst envelope temperature or by glowing particles of catalyst which have become detached. The Oxoid Catalyst design protects the customers from both of these possibilities.

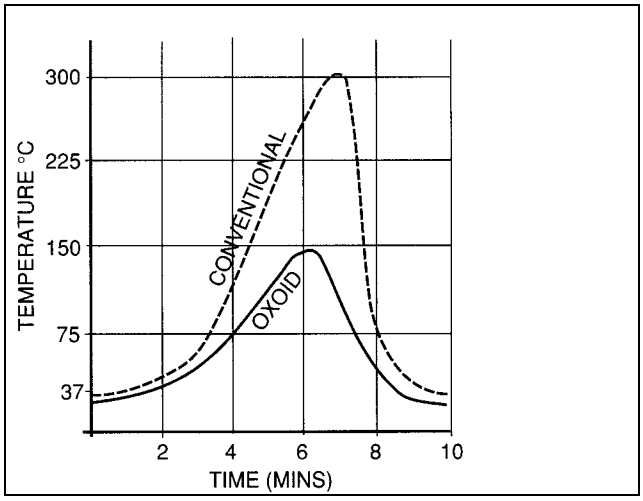
How the Oxoid Anaerobic Catalyst BR42 helps the microbiologist

Features

1 Maximum working temperature is approximately half that of other catalysts.
2 Double wrapped in perforated aluminium foil and fine stainless steel mesh.
3 Four grams of catalyst (more than 1 gram/litre airspace) is provided.

Benefits

1 Safety in use because the maximum operating temperature is lower than the flash point of hydrogen gas.
2 Acts as an efficient heat sink
3 Method of wrapping minimises the likelihood of small particles falling into jars thus ensuring extra safety in use.
4 Helps to create anaerobic conditions quickly.



THE OXOID ANAEROBIC INDICATOR BR55

The Oxoid Anaerobic Indicator BR55 consists of a cotton strip impregnated with a redox indicator solution enclosed in a laminated foil envelope. This formulation and a pure cotton strip gives a reproducible redox colour change in a shorter time than similar products that are available. Use of the Oxoid Anaerobic Indicator will support the evidence of pressure changes which occur with active catalysts and ensure that the absence of growth does not reflect poor anaerobic incubation.

How the Oxoid Anaerobic Indicator BR55 helps the microbiologist

Features

1 Changes from red to white.

2 Improved sensitivity to detect lower levels of oxygen than has previously been achievable.

Benefits

1 Indicates when true anaerobiosis has been achieved.

2 Indicates better anaerobic conditions.

References

1 Patent application 54354/7 developed by Don Whitley Scientific Limited.

2 United Kingdom Department of Health and Social Security. February 1979.

THE ATMOSPHERE GENERATION SYSTEM

In 1993, Oxoid launched a new range of innovative products under the title of Atmosphere Generation System (AGS). These novel products are safer (no hydrogen produced) and more convenient (no water to add). They include a new jar of advanced design – Anaerojar – in which it is not necessary to use a catalyst.

This range has frequently been extended and now consists of the following products:

Anaerobic Indicator	Code BR055B	Anaerobic Atmosphere Generation System
AnaeroGen™ (for 2.5 litre jar)	Code AN025A	
AnaeroGen™ (for 3.5 litre jar)	Code AN035A	
AnaeroGen Compact™ (for use with plastic pouches)	Code AN010C	
CampyGen™ (for 2.5 litre jar)	Code CN025A	Atmosphere Generation for Micro-aerophilic organisms
CampyGen™ (for 3.5 litre jar)	Code CN035A	
CampyGen Compact (for use with plastic pouches)	Code CN020C	
CO_2Gen™ (for 2.5 litre jar)	Code CD025A	Atmosphere Generation for CO_2 dependent organisms
CO_2Gen Compact (for use with plastic pouches)	Code CD020C	
AnaeroJar™	Code AG025A	

A range of accessories for the Compact products and the AnaeroJar completes the Atmosphere Generation System.

ANAEROGEN

Code: AN25 & AN35

Description

Where an AnaeroGen sachet is placed in a sealed jar, the atmospheric oxygen in the jar is rapidly absorbed with the simultaneous generation of carbon dioxide. This novel method differs from those commonly used in that the reaction proceeds with no evolution of hydrogen, and therefore does not require a catalyst. Furthermore, no addition of water is needed to activate the reaction.

When used as directed, the AnaeroGen sachet will reduce the oxygen level in the jar to below 1% within 30 minutes. The resulting carbon dioxide level will be between 9% and 13%.

AnaeroGen was used in methodology for detecting bifidobacteria in meat and meat products in an investigation into the suitability of these organisms as indicators of faecal contamination.

Components
Each box contains:

10 AnaeroGen paper sachets which are individually foil packed.

1 Product Insert.

The active component within each AnaeroGen sachet is ascorbic acid.

Precautions

This product is for *in-vitro* use only.

As soon as the AnaeroGen paper sachet is exposed to the air, the reaction will start. It is therefore essential that the paper sachet is placed in the jar and the jar sealed within one minute.

The reaction of the ascorbic acid with oxygen is exothermic. However, the temperature of the AnaeroGen paper sachet will not exceed 65°C.

Storage
Store at 2–25°C. Under these conditions, the AnaeroGen sachets will retain their reactivity until the expiry date declared on the outer box and on the foil sachet.

Directions
AN35 is designed for use in 3.5 litre jars. It is therefore suitable for the Oxoid Anaerobic Jar HP11 and for other jars of similar capacity.

AN25 is designed for use in 2.5 litre jars such as the new Oxoid AnaeroJar AG25 and other jars of similar capacity.

1 Place the inoculated media plates in the appropriate anaerobic jar. Disposable plastic petri dishes should be of the vented variety to aid gas transfer between the interior and exterior of the plates.
2 Tear open an AnaeroGen foil sachet at the tear-nick indicated, and remove the AnaeroGen paper sachet from within.
3 Immediately place the AnaeroGen paper sachet in the appropriate clip on the plate carrier within the jar.

   N.B. The AnaeroGen paper sachet will become warm to touch on exposure to air.
4 Close the jar lid immediately.

   N.B. The time taken between opening the foil sachet and sealing the jar should not exceed 1 minute. Extended exposure will result in loss of reactivity, and full anaerobic conditions may not be achieved in the jar.
5 After the appropriate incubation period remove the plates and examine for the presence of anaerobes. If the plates require re-incubation then a fresh AnaeroGen sachet must be used following steps 2–5 described above.
6 After incubation, the exhausted AnaeroGen sachet should be discarded with the appropriate laboratory waste.

Control Testing
It is recommended that an OXOID Anaerobic Indicator (BR55) is also used in the jar as a visual check that anaerobic conditions have been achieved and maintained.

The user should check their Anaerobic system periodically for its ability to provide adequate conditions for the growth of appropriate bacteria. The following strains are recommended:

Clostridium novyii ATCC® 9690 growth

Micrococcus luteus ATCC® 9341 no growth

Disposal
On removal from the jar after incubation, the AnaeroGen paper sachet will retain a small amount of reactivity and will warm up. The sachets should therefore be allowed to cool at room temperature prior to disposal alongside the appropriate laboratory waste.

Reference

1 Beerens H. (1998) *Int. J. Food Microbiol. 40. 203–207.*

ANAEROGEN™ COMPACT

Code: AN010C

AnaeroGen Compact is a simple system for the anaerobic incubation of up to 4 petri dishes or an identification panel.

Description
The system consists of a plastic pouch and a paper gas generating sachet. The paper sachet contains ascorbic acid and activated carbon which react on contact with air. Oxygen is rapidly absorbed and carbon dioxide is produced. When the paper sachet is placed in a sealed plastic pouch, this reaction will create ideal atmospheric conditions for the growth of anaerobes. It proceeds with **no** evolution of hydrogen, and therefore does **not** require a catalyst. **No** addition of water is necessary to activate the reaction. This gives the system many advantages over the commonly used borohydride systems with increased safety and convenience.

When used as directed, the AnaeroGen Compact sachet will reduce the oxygen content in the pouch to below 1% within 30 minutes. The resulting carbon dioxide content will be between 8% and 14%. The level of carbon dioxide will depend on how many plates are placed in the pouch. AnaeroGen Compact has been designed for use with 1–4 plates.

Components
Each box contains:

10 AnaeroGen Compact paper sachets, individually wrapped in aluminium foil

10 Plastic Pouches

1 Product Insert

The active components within each AnaeroGen Compact sachet are ascorbic acid and activated carbon.

Materials Required but not Provided
AnaeroGen Compact Sealing Clips (AN005C).

Precautions
This product is for *in vitro* use only.

The AnaeroGen Compact paper sachet will become active on contact with air. It it therefore essential that the paper sachet is placed into the pouch and the

pouch sealed within one minute.

The reaction of the ascorbic acid with oxygen is exothermic. However, the temperature of the AnaeroGen Compact paper sachet will not exceed 65°C.

This temperature will only be maintained while anaerobic conditions are being achieved. Once the oxygen in the pouch has been absorbed, the temperature within the pouch will return to ambient temperature.

Storage
Store at 2–25°C. Under these conditions, the AnaeroGen Compact sachets will retain their activity until the expiry date declared on the outer box and on the foil wrapped sachet.

Directions
1 Place the inoculated media plates or identification panel in the plastic pouch provided. Disposable plastic petri dishes should be of the vented variety to aid gas transfer between the interior and exterior of the plates.
2 Tear open an AnaeroGen Compact foil sachet at the tear-nick indicated. Remove the AnaeroGen Compact paper sachet from within.
3 Immediately place the AnaeroGen Compact paper sachet in the plastic pouch.

   N.B. The AnaeroGen Compact paper sachet will become warm to the touch on exposure to air.
4 Expel excess air from the plastic pouch. Seal the plastic pouch immediately with the AnaeroGen Compact clip (AN005C).

   N.B. the time taken between opening the foil sachet and sealing the plastic pouch should not exceed 1 minute. Extended exposure will result in loss of reactivity, and full anaerobic conditions may not be achieved in the pouch.
5 Incubate appropriately.
6 After the incubation period remove the plates or ID panel and examine for the presence of colonies or biochemical reaction. If the plates require re-incubation then a fresh AnaeroGen Compact sachet must be used following steps 2–5 described above.

   N.B. The plates may be initially inspected through the transparent plastic pouch. If the bag is not opened, a fresh AnaeroGen Compact sachet is not required for re-incubation.
7 After incubation, the exhausted AnaeroGen Compact paper sachet and plastic pouch should be sterilised and discarded with the non-hazardous laboratory waste.

Control Testing
It is recommended that OXOID Anaerobic Indicator (BR055B) is also used in the plastic pouch as a visual check that anaerobic conditions have been achieved and maintained.

The user should check their anaerobic technique periodically for its ability to provide adequate conditions for the growth of anaerobic bacteria. The following strains are recommended:

Clostridium novyii	ATCC® 9690 growth
Micrococcus luteus	ATCC® 9341 no growth

Disposal
On removal from the pouch after incubation, the AnaeroGen Compact paper sachet will retain a small amount of activity and become warm. The sachets should be allowed to cool to room temperature prior to sterilisation and disposal with the non-hazardous laboratory waste.

ANAEROJAR

Code: AG25

Description
The 2.5 litre Oxoid AnaeroJar is an important addition to the Oxoid range of Atmosphere Generation Products. The jar is designed for use with the 2.5 litre AnaeroGen/CampyGen sachet.

Important features include:

- No catalyst required.
- Polycarbonate base which is secured to the lid by 4 clips.

  These clips are designed to allow venting in the unlikely event of a positive pressure build-up occurring i.e. by allowing lid to lift and reseal to maintain correct conditions.
- A carrying handle for the safe transportation of the jar from bench to incubator.
- Vacuum Relief Screw to overcome any vacuum which may occasionally occur.

Operating Instructions
Note
Before use check:

a. 'O' ring is correctly seated

b. The vacuum relief screw is in the closed position.

1 Place inoculated plates into the plate carrier. Disposable plastic petri dishes should be of the vented variety to aid gas transfer between interior and exterior of the dishes.
2 When using the anaerobic system (i.e. AN25) prepare the Oxoid Anaeraobic Indicator (BR55) by cutting and exposing 10mm of the fabric strip, insert into the smaller, upper clip on the dish carrier.
3 Lower the carrier into the polycarbonate base.
4 Tear open an AnaeroGen/CampyGen/CO_2Gen sachet at the tear-nick indicated, and remove the paper sachet from within.
5 Immediately place the paper sachet in the appropriate clip in the plate carrier within the jar (see Technical insert).
6 Having inserted the sachet into the carrier immediately place the lid on the jar, making sure the 'O' ring is in place. Secure the clips with fingers shown in figure[1]. Repeat this process with each of the four clips to properly secure the lid.
7 Use carrying handle situated on the lid to transport jar to the incubator.
8 The anaerobic indicator will change from pink to white giving a visual indication of anaerobiosis.

9 Remove jar after the appropriate incubation period and open lid by carefully depressing the clips to release the jar lid from the base. Excessive downward pressure on the clips should be avoided.

10 Occasionally a slight vacuum may occur after anaerobiosis producing a negative pressure, resulting in resistance to the removal of the lid (after release of the clips). This is overcome by placing an appropriate object such as a small coin into the screw and turning anticlockwise allowing inlet of air. It is important, however, to ensure the valve is resealed, by turning clockwise, prior to further use.

Precautions

1 THE JAR IS DESIGNED TO BE USED WITH ANAEROGEN/CAMPYGEN/CO_2GEN AND MUST NOT BE USED WITH GAS GENERATING SYSTEMS REQUIRING THE USE OF CATALYST (BR38), WHICH WITHOUT CATALYST WOULD RESULT IN A POTENTIALLY EXPLOSIVE H_2/O_2 GAS MIXTURE.

2 The jar should not be autoclaved.

Cleaning and Disinfection

Note

Disposable gloves should be worn throughout the following operations.

Internal surface should be cleaned and disinfected with a compatible proprietary disinfectant made up to manufacturer's recommended instructions.

Disinfectants such as sodium hypochlorite, phenolic compounds, methyl alcohol and chloroform should be avoided as they will damage the surface of the jar.

It is imperative the jar is properly disinfected if it is necessary to return it to Oxoid.

Routine Maintenance and Checking

1 Lid and outer surface can be cleaned and dried with a soft tissue.

2 Regularly check integrity of the 'O' ring. Replace if there are any signs of deterioration such as splitting. Do not allow grease/organic solvents to come into contact.

3 Ensure that the jar is dry before use. Store in a suitable environment as excess moisture may quench reaction. The appearance of condensation during use is normal.

CAMPYGEN

Code: CN25 & CN35

Description

When a CampyGen sachet is placed in a sealed jar, the atmospheric oxygen in the jar is rapidly absorbed with the simultaneous generation of carbon dioxide, producing the appropriate microaerobic conditions. This novel method differs from those commonly used in that the reaction proceeds with no evolution of hydrogen, and therefore does not require a catalyst. Furthermore, no addition of water is needed to activate the reaction.

An evaluation which compared CampyGen with the evacuation/replacement method and gas generating envelopes showed CampyGen to be effective.

Components

Each box contains:

10 CampyGen paper sachets which are individually foil packed.

1 Product Insert

The active component within each CampyGen sachet is ascorbic acid.

Precautions

This product is for *in vitro* use only

As soon as the CampyGen paper sachet is exposed to air, the reaction will start. It is therefore essential that the paper sachet is placed in the jar and the jar sealed within one minute.

The reaction of the ascorbic acid with oxygen is exothermic. However, the temperature of the CampyGen paper sachet will not exceed 65°C.

Storage

Store at 2–25°C. Under these conditions, the CampyGen sachets will retain their reactivity until the expiry date declared on the outer box and on the foil sachet.

Directions

CN25 is designed for use in 2.5 litre jars including the new Oxoid AnaeroJar.

CN35 is designed for use in 3.5 litre jars.

1 Place the inoculated media plates in the appropriate jar. Disposable plastic petri dishes should be of the vented variety to aid gas transfer between the interior and exterior of the plates.

2 Tear open the CampyGen foil sachet at the tear-nick indicated, and remove the CampyGen paper sachet from within.

3 Immediately place the CampyGen paper sachet in the appropriate clip on the plate carrier within the jar.

N.B. The CampyGen paper sachet will become warm to the touch on exposure to air.

4 Close the jar lid immediately.

N.B. The time taken between opening the foil sachet and sealing the jar should not exceed 1 minute. Extended exposure will result in loss of reactivity, and microaerobic conditions may not be achieved in the jar.

5 After the appropriate incubation period remove the plates and examine for the presence of Campylobacter. If the plates require re-incubation then a fresh CampyGen sachet must be used following steps 2–5 described above.

6 After incubation, the exhausted CampyGen sachet should be discarded with the appropriate laboratory waste.

Control Testing

The user should check their microaerobic system periodically for its ability to provide adequate conditions for the growth of appropriate bacteria. The following strain can be used for this purpose.

Campylobacter jejuni ATCC® 33291

Disposal
On removal from the jar after incubation, the CampyGen paper sachet may retain a small amount of reactivity and will warm up. The sachets should therefore be allowed to cool to room temperature on an inert surface prior to disposal with the laboratory waste.

Reference
1 Bolton F. J., Wareing D. R. A. and Sails A. D. (1997) *Eur. J. Clin. Microbiol. Inf. Dis. 16. 839–842.*

CAMPYGEN COMPACT

Code: CN020C

Description
CampyGen Compact for 1 or 2 petri dishes, is a simple system for generating microaerobic conditions. The system consists of a plastic pouch and sealing clip and a paper gas generating sachet. The paper sachet contains ascorbic acid which reacts on contact with air to produce the microaerobic conditions for the growth of microaerophilic organisms.

Components
20 CampyGen Compact paper sachets, individually wrapped in foil

1 product leaflet

Materials Required but not Provided
Sealing Clips (AN005C)

Plastic Pouches (AG020C).

Precautions
This product is for *in vitro* use only.

The CampyGen Compact paper sachet will become active on contact with air. It is essential that the plastic pouch is sealed within one minute of exposing the paper sachet to the air.

The reaction of ascorbic acid with oxygen is exothermic. However, the temperature of the CampyGen Compact paper sachet will not exceed 65°C.

Storage
Store at 2–25°C. Under these conditions, the CampyGen Compact sachets will retain their activity until the expiry date given on the outer box and on the foil wrap of the sachets.

Directions
1 Place 2 inoculated plates in a plastic pouch. Disposable plastic petri dishes should be of the vented variety to aid gas transfer between the interior and exterior of the plates. If only one plate is to be inoculated, an uninoculated plate should also be placed in the plastic pouch to prevent further activity as the volume of O_2 and CO_2 is critical.
2 Tear open a CampyGen Compact foil sachet at the tear-nick indicated. Remove the CampyGen Compact paper sachet from within.
3 Immediately place the paper sachet in the plastic pouch with the plates.

N.B. The paper sachet will become warm to the touch on exposure to air.

4 Expel excess air from the plastic pouch. Seal the plastic pouch immediately with a sealing clip. The time taken between opening the foil sachet and sealing the plastic pouch should not exceed 1 minute.
5 Incubate appropriately.
6 After the incubation period, remove the plates and examine for the presence of colonies. If the plates require re-incubation, a fresh CampyGen Compact sachet must be used following steps 2–5 described above.

N.B. The plates may be initially inspected through the transparent plastic pouch. If the bag is opened, a fresh CampyGen Compact sachet is required for re-incubation.

7 After incubation, the exhausted CampyGen Compact paper sachet and plastic pouch should be sterilised and discarded with the non-hazardous laboratory waste.

Control Testing
The user should check their technique periodically for their ability to provide adequate conditions for the growth of microaerophilic bacteria. *Campylobacter jejuni* (ATCC® 33291) may be used for this purpose.

Disposal
On removal from the pouch after incubation, the CampyGen Compact paper sachet will retain a small amount of activity and become warm. The sachets should be allowed to cool to room temperature prior to sterilisation and disposal with the non-hazardous laboratory waste.

CO_2GEN

Code: CD025A

Description
CO_2Gen is designed for the generation of a carbon dioxide-rich atmosphere within a gas jar. The paper sachet contains ascorbic acid which reacts on contact with air to produce a level of approximately 6% carbon dioxide within a 2.5 litre gas jar such as the Oxoid AnaeroJar (AG025A). The final concentration of oxygen is 15%.

Components
10 CO_2Gen paper sachets, individually wrapped in foil

1 product leaflet

Materials Required but not Provided
2.5 litre gas jar (Oxoid AnaeroJar AG025A).

Precautions
This product is for *in vitro* use only.

The CO_2Gen paper sachet will become active on contact with air. It is therefore essential that the paper sachet is placed in the jar and the jar sealed within one minute.

The reaction of the ascorbic acid with oxygen is exothermic. However, the temperature of the CO_2Gen paper sachet will not exceed 65°C.

Storage
Store at 2–25°C. Under these conditions, the CO_2Gen sachets will retain their activity until the expiry date given on the outer box and on the foil wrap of the sachets.

Directions
1 Place inoculated media plates in a 2.5 litre gas jar. **Do not use a 3.5 litre jar.** Disposable plastic petri dishes should be of the vented variety to aid gas transfer between the interior and exterior of the plates.
2 Tear open a CO_2Gen foil sachet at the tear-nick indicated. Remove the sachet from within.
3 Immediately place the paper sachet in the 2.5 litre gas jar.

   N.B. The paper sachet will become warm to the touch on exposure to air.
4 Seal the jar immediately. The time taken between opening the foil sachet and sealing the jar should not exceed 1 minute.
5 Incubate appropriately.
6 After the incubation period, remove the plates and examine for the presence of colonies. If the plates require re-incubation, a fresh CO_2Gen sachet must be used following steps 2–5 described above.
7 After incubation, the exhausted CO_2Gen paper sachet should be sterilised and discarded with the non-hazardous laboratory waste.

Disposal
On removal from the jar after incubation, the CO_2Gen paper sachet will retain a small amount of activity and become warm. The sachets should be allowed to cool to room temperature prior to sterilisation and disposal with the non-hazardous laboratory waste.

CO_2GEN COMPACT

Code: CD020C

Description
CO_2Gen Compact is a simple system for the generation of a carbon dioxide-enriched atmosphere for the incubation of 2 petri dishes. The system consists of a plastic pouch and sealing clip and a paper gas generating sachet. The paper sachet contains ascorbic acid which reacts on contact with air to produce an atmosphere which contains approximately 6% carbon dioxide. The final concentration of oxygen is 15%.

Components
20 CO_2Gen Compact paper sachets, individually wrapped in foil

1 product leaflet

Materials Required but not Provided
Sealing Clips (AN005C)

Plastic Pouches (AG020C)

Precautions
This product is for *in vitro* use only.

The CO_2Gen Compact paper sachet will become active on contact with air. It is therefore essential that the paper sachet is placed in the pouch and the pouch sealed within one minute.

The reaction of the ascorbic acid with oxygen is exothermic. However, the temperature of the CO_2Gen Compact paper sachet will not exceed 65°C.

Storage
Store at 2–25°C. Under these conditions, the CO_2Gen Compact sachets will retain their activity until the expiry date given on the outer box and on the foil wrap of the sachets.

Directions
1 Place 2 inoculated media plates in a plastic pouch. Disposable plastic petri dishes should be of the vented variety to aid gas transfer between the interior and exterior of the plates. If only one plate is to be inoculated, an uninoculated plate should also be placed in the plastic pouch.
2 Tear open a CO_2Gen Compact foil sachet at the tear-nick indicated. Remove the CO_2Gen Compact paper sachet from within.
3 Immediately place the paper sachet in the plastic pouch.

   N.B. The paper sachet will become warm to the touch on exposure to air.
4 Expel excess air from the plastic pouch. Seal the plastic pouch immediately with a sealing clip. The time taken between opening the foil sachet and sealing the plastic pouch should not exceed 1 minute.
5 Incubate appropriately.
6 After the incubation period, remove the plates and examine for the presence of colonies. If the plates require re-incubation, a fresh CO_2Gen Compact sachet must be used following steps 2–5 described above.

   N.B. The plates may be initially inspected through the transparent plastic pouch. If the bag is not opened, a fresh CO_2Gen Compact sachet is not required for re-incubation.
7 After incubation, the exhausted CO_2Gen Compact paper sachet and plastic pouch should be sterilised and discarded with the non-hazardous laboratory waste.

Disposal
On removal from the pouch after incubation, the CO_2Gen Compact paper sachet will retain a small amount of activity and become warm. The sachets should be allowed to cool to room temperature prior to sterilisation and disposal with the non-hazardous laboratory waste.

NOTE

In addition Oxoid manufacture a wide range of media for the transport, culture, selective isolation and susceptibility testing of anaerobic bacteria.

The Microbiology Laboratory that is equipped with Oxoid Anaerobic Systems will have:
- Highly flexible systems that can cope equally well with Gas Generating Envelopes or Gas Cylinders.
- Effective systems that will provide rapid production of the atmosphere within the jar and ensure growth even of those anaerobes which may have been damaged in transit to the laboratory.

- The safest Anaerobic Systems.
- Advanced systems which contain all the facilities recommended by leading microbiologists in the field of anaerobic bacteriology.
- The best systems to ensure high isolation rates of even the most fastidious anaerobes.

8
BLOOD CULTURE SYSTEMS

November 1998

BLOOD CULTURE SYSTEMS

INTRODUCTION

The rapid and reliable detection of organisms in the blood of patients is important to help guide the optimal treatment of septicaemia and infective endocarditis. It also aids in the diagnosis of infective cases of pyrexia of unknown origin. The importance of the detection, isolation, identification and antimicrobial susceptibility testing of these micro-organisms is underscored by the correlation between the appropriateness of antimicrobial therapy and the outcome of the septic episode. Microbiologists must, therefore, select the optimal procedures for the rapid detection and isolation of the etiological agent.

Blood culture systems have diversified since the 1960s when mainly ''home made'' systems were the only option. Commercially prepared broth cultures, with sodium polyanetholesulfonate added to counteract the effect of phagocytes and complement in blood were the first innovation. Then the use of biphasic media was extended from Castenada's method for the isolation of *Brucella* sp., to routine culture of other bacteria and fungi. Semi-automated methods followed with manometric, radiometric, infrared spectronomy, CO_2 detection, bioluminescence, electrical impedance and fluorescent detection methods being employed.

Septicaemia
The terminology for the presence of micro-organisms in the blood uses the name of the organism plus the suffix 'aemia': bacteraemia, viraemia, fungaemia etc. Confusion can arise by the interchangeable use of bacteraemia and septicaemia. Bacteraemia is the presence of organisms in the blood stream. However, septicaemia implies the multiplication of organisms with clinical symptoms and signs of disease[1,2].

The most frequently isolated bacteria are Gram-negative bacilli, followed by pyogenic cocci (e.g. staphylococci, streptococci) and anaerobes[3,4]. Viruses and fungi may also be transported in the blood stream to seed other organs. Some protozoa are especially adapted to life in the blood circulation system and carry out parts of their life cycle within it (e.g. agents of malaria, leishmaniasis and trypanosomiasis).

In normal circumstances the blood stream is a relatively inhospitable part of the body for micro-organisms. Blood contains many powerful antimicrobial systems, including leucocytes, immunoglobulins and complement. These chemical barriers act within the moving blood stream, e.g. antibiotics and complement can attach to circulating bacteria. Conversely, cellular defences, such as neutrophils, act efficiently only after the blood has delivered them to a suitable site in another tissue. The blood stream can carry opsonised bacteria to the spleen, liver or bone marrow. Reticulo-endothelial cells lining these channels can effectively remove vast numbers of opsonised bacteria from the circulation.

In a normal immunocompetent person the blood will normally be sterile. However, bacterial 'normal flora' can 'spill over' mechanical barriers such as skin or mucosa. These organisms are usually non-pathogenic to the host and are quickly cleared away without any ill effect.

Establishment of a clinically significant blood stream infection requires one or more of the following conditions:

1 Introduction of a large enough inoculum of bacteria to overwhelm normal defences.
2 Pre-existing impairment of defence mechanisms.
3 Adaptation of the invading organisms to survive in the blood.

Modern therapeutic and diagnostic techniques, whilst giving enormous advantages to their specialist areas, unfortunately can facilitate entry of bacteria into the blood stream and render the host less able to remove organisms. The following factors may weaken or interfere with host defences.

Invasive techniques
Catheters of all kinds, and other medical devices, often by-pass natural host barriers and permit bacteria to enter the blood stream.

Immunosuppressive therapy and diseases
Drugs given to combat malignancies, inflammatory diseases and for organ transplantation are being given to an even larger number of patients. Patients with the Human Immunodeficiency Virus (HIV) have little or no resistance to bacterial infection.

Supportive measures
Patients are kept alive for long periods despite severe incurable underlying disease.

Antibiotics
High doses and use of multiple agents promote the selection of resistant organisms.

The clinical manifestation of septicaemia are usually due to toxic bacterial products, the host response, or both. With Gram-negative septicaemia the major problem is endotoxin, this is the lipopolysaccharide of the outer membrane of these organisms[5]. The lipid A portion is responsible for a chain of reactions, activation of other molecules including TNF, IL-1 and complement which contribute to the toxic shock experienced in patients with severe Gram-negative septicaemia. Gram-positive organisms involve a lesser likelihood of shock, but some do produce endotoxins which, in the case of *Staphylococcus aureus* have been implicated in producing the manifestations of toxic shock[1,5,6].

Studies have indicated that there is a correlation between appropriate antimicrobial therapy and outcome of the septic episode[7,8]. Although many factors influence the initial selection of an antimicrobial agent, such as underlying disease or conditions, immune status and probable cause of infection, a successful outcome is enhanced by use of antimicrobial agents to which the causative organism is susceptible[9]. The clinical microbiology laboratory's ability to rapidly detect, isolate, identify and provide an antimicrobial susceptibility pattern is obviously of the utmost importance[10].

Endocarditis
Infective endocarditis is the disease caused by infection of the endothelial surface of the heart, most often located on one of the valves. Before antibiotic therapy was available endocarditis was described as acute, sub-acute or chronic, defined by the length of time from onset to death. Predisposition to infective endocarditis in the past was mainly chronic rheumatic heart disease. This is now much less prevalent but has been replaced by other cardiac conditions: congenital heart disease, mitral valve prolapse and degenerative valvular disease in the elderly.

Infective endocarditis is often caused by members of the normal oropharyngeal flora. The organisms enter the blood stream through minor abrasions caused by toothbrushing, flossing etc. About 1 in every 5 patients with an endocarditis caused by an alpha haemolytic streptococcus have had dental work shortly before the onset of the disease. Less commonly organisms from faecal and vaginal tracts may enter the blood stream e.g. *Enterococcus* spp.

Staph. aureus is the most common cause of acute endocarditis in patients with normal heart valves, other organisms include groups B and D streptococci, pneumococci and gonococci.

Infective endocarditis is almost always preceded by bacteraemia, except when it is caused by the contamination of prosthetic valves at time of surgery. *Staph. epidermidis* is the most frequent infecting organism. Gram-positive organisms are the most common cause of endocarditis as they tend to adhere more easily to collagen, fibrin, platelets and heart valve surfaces. Underlying valvular disease contributes to bacterial colonisation in several ways:

Damaged or incompetent valves cause eddies and turbulence, which leads to deposits of fibrin and platelets on exposed collagen.

On a damaged endocardium bacteria may shelter in vegetations within which they can freely multiply. They may re-seed the blood stream, leading to a continuous bacteraemia and possible metastatic infections in other sites.

The key to diagnosis of infective endocarditis is blood culture. This is essential as the aetiology and subsequently the antimicrobial susceptibility pattern of each case vary considerably. High doses of bactericidal antibiotics effective against the infectious agent for a prolonged length of time is the usual regime. Therefore the rapid isolation, identification and susceptibility pattern of the causative organism is of optimal importance[1].

Bacteraemia in children and neonates
Blood culture specimens from young children are not necessarily processed optimally when cultured in the same fashion as specimens from older patients[11]. Blood stream infections and therefore blood culture specimens differ in children in four areas; firstly, the concentration of micro-organisms in blood is usually higher than that found in adults; secondly, the volume of blood is, by necessity, considerably less from children; thirdly, some micro-organisms, (e.g. *Haemophilus influenzae* type b, *Streptococcus pneumoniae, Neisseria meningitidis* cause more frequent infection in children than adults[12]. Finally, polymicrobial or anaerobic bacteraemia is much rarer in children than in adults[11].

Most neonatal patients have only one blood sample drawn with a volume ranging from 0.5–1.5ml. It is important to use a blood culture system that provides a sensitive method for the detection of neonatal septicaemia[13].

Collection of Specimens
The physical collection of blood can pose many problems. The patient's skin must be adequately disinfected to avoid members of the normal skin flora being incorporated into the culture system. This is especially important now that these bacteria are being isolated in increasing numbers, as infectious agents, from immunocompromised patients. Separate swabs containing 70% ethanol or isopropyl alcohol, to clean the patient and the rubber caps of the culture bottles have been recommended[14].

It has been shown that trained teams of phlebotomy staff can reduce contamination rates to 2–3% in adults, as opposed to using medical or nursing staff[14]. Contamination rates are higher in children, this could be due to uncooperative patients and the skin of such patients with profuse respiratory secretions and diarrhoea is likely to harbour high bacterial counts. One problem with phlebotomy teams is cost – to whom are they responsible and who foots the salary bill? The cost must be weighed against the incidences and the cost of improperly collected specimens, excessive collection of blood and contaminated bottles[15].

With an increasing number of patients with temporary or permanent intra-vascular lines that often serve as a major access site to blood for a variety of therapeutic and monitoring purposes, there has been an increase in blood collection for culture from these sites. Contamination of blood, e.g. by *Staph. epidermidis*, may occur when drawn through these lines, contamination has also been shown when the collection is distributed into other testing systems, e.g. ESR bottles, before inoculation into the blood culture bottles. It is recommended that blood for culture is always collected separately[14].

It has been shown that differential quantitative cultures of blood drawn simultaneously from a suspected infected intra-vascular device and a peripheral vein, is accurate in predicting catheter-related sepsis. Usually a four fold increase between the two blood samples is seen[13,16].

The timing of blood collection in endocarditis is probably unimportant, but in most other conditions bacteraemia is intermittent, being related to the fevers and rigors which occur 30–60 minutes after entry of the organisms into the blood stream. Ideally, cultures should be taken before antimicrobial therapy has started. Up to three sets are usually adequate to establish septicaemia[8].

A series of studies have shown that in bacteraemic adults the probability of blood cultures being positive increases markedly when larger volumes of blood are

cultured[8,17]. Culturing 10ml instead of 5ml can increase the isolation rate by about 15%, from 20ml the rate was 35% greater than 5ml[14]. When a commercial blood culture system is used the manufacturer's recommendations should be followed, but with some systems this could involve culturing an inadequate amount of blood. With children, because the total blood volume is much less than in adults, it is not feasible to culture large volumes of blood. However, many colony counts in children tend to be higher than in adults and satisfactory results can be obtained when 1–5ml of blood are cultured.

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First published in the *Newsletter of the British Society of Microbial Technology*.

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OXOID SIGNAL BLOOD CULTURE SYSTEM

The following section describes briefly the Oxoid SIGNAL Blood Culture System, the principles of its function and the equipment required for its optimal performance. For full details of the usage of the System the product insert should be consulted.

Principle of the Test

Blood samples are collected from patients, using strict aseptic technique and sterile equipment. The samples are inoculated into the blood culture bottles and mixed with the medium.

The formulation of the medium encourages the growth of aerobic, anaerobic and micro-aerophilic organisms. The medium is also designed to create pressure in the sealed bottle when organisms are growing.

The detection of positive pressure is by means of a growth indicator device which is connected to the bottle after the blood sample is added. A positive pressure in the bottle displaces a quantity of blood/broth mixture into the chamber as a sign of microbial activity.[2,3,4,5]

A positive result is indicated when the blood/broth mixture rises above the green locking sleeve of the growth indicator device.

Medium Composition

Typical formulation (European Patent 0124193 Al)

	gm/litre
Tryptone Soya Broth	10.0
Gelatin peptone	10.0
Yeast extract	5.0
Meat extract	5.0
Sodium chloride	8.0
Potassium nitrate	2.0
Glucose	1.0
L-arginine	1.0
Sodium pyruvate	1.0
Gelatin	1.0
Sodium thioglycollate	0.5
Cysteine HCl	0.4
Sodium bicarbonate	0.4
Phosphate buffer	0.3
Sodium polyanethol sulphonate	0.3
Dithiothreitol	0.2

Adenine sulphate	0.01
Sodium succinate	0.01
Ammonium chloride	0.008
Magnesium sulphate	0.008
Menadione	0.005
pH 7.0	

Sodium polyanethol sulphonate (SPS), 0.03% is added because it inhibits clotting,[6] neutralises the bactericidal effect of human serum[7], prevents phagocytosis[8] and partially inactivates certain antibiotics (streptomycin, kanamycin, gentamicin and polymyxin B).[9,10] SPS may be inhibitory to some strains of *Peptostreptococcus anaerobius, Neisseria meningitidis* and *N. gonorrhoeae;* therefore gelatin is added to the medium to neutralise this inhibition.[11,12] When human blood is added to this medium, CO_2 produced can be detected at 2.5 to 5% v/v in the bottle head-space.[5]

Materials required but not provided

1 Sterile syringe or other means of obtaining blood.
2 Alcohol solutions, or other suitable skin disinfection material.
3 Culture media and other equipment for subcultures.
4 Incubator equipment to maintain 36° ± 1°C.
5 Orbital shaker (for optimal results). Available from Oxoid Ltd, Code BC 301 (110 to 120 volt), BC 302 (220 to 240 volt)

or

6 Incubator shaker BC 107 (110 to 120 volt), BC 108 (220 to 240 volt). Allows continuous visual monitoring of the system.

Components of the System

1 A sealed blood culture bottle containing 80ml of broth medium. 20 bottles per pack, product code BC 102.
2 A sterile growth indicator device which is vented through a 0.2 micron hydrophobic membrane. 20 per pack, product code BC 101.

Method of Use (for full details see current product insert)

A. Inoculation Procedure

1 Examine the bottle of broth before taking the blood sample and discard it if any evidence of contamination can be seen.
2 Prepare the bottle for inoculation before taking the blood sample. Remove the green plastic 'flip-off' cap and disinfect the exposed part of the rubber stopper.
3 Aseptically inject a maximum volume of 10ml of blood through the central ring of the rubber stopper. (The partial vacuum in the bottle will accept 12ml of blood.)
4 Thoroughly mix the blood with the broth in the bottle.
5 Write the patient's name and identification details on the bottle label.
6 Immediately transfer the inoculated blood culture bottle to the laboratory. In the event of the laboratory being closed or transportation being delayed, the bottle should be incubated at 36° ± 1°C, and the 'Laboratory Procedure', detailed below, carried out at the earliest opportunity (within 24 hours).

B. Laboratory Procedure

1 Place the inoculated bottle in an incubator at 36° ± 1°C for approximately 1 hour.
2 Remove from the incubator and place the bottle in an incubation tray (BC 104).
3 Remove the growth indicator device from its sterile package and ensure that the needle and cap are fully tightened. (Hold the clear plastic body of the device with the covered needle pointing downwards. Tighten the needle by turning the needle cover anti-clockwise. Tighten the cap by turning it clockwise.)
4 Disinfect the rubber stopper of the bottle by swabbing, e.g. with alcohol.
5 Slide the plastic shield from the needle. Do not touch the needle.
6 Aseptically insert the needle through the centre of the rubber stopper. Push the needle shaft as far as it will go through the rubber stopper.
7 Slide the green locking sleeve of the growth indicator device downwards until it fully locks on to the neck of the blood culture bottle. Press down the chamber to ensure full contact with the rubber seal of the bottle.
8 For optimal results shake the system for approximately 24 hours at 150 orbits/minute, using a shaker placed in the incubator, or a bench top integrated shaker/incubator, at 36° ± 1°C. (If use of a shaker in the first 24 hours is impossible the system should be manually shaken as often as possible (at least 4 times) during this period.)
9 Examination of the system for a positive result should be carried out at least twice daily.
10 At the end of the 24 hour period, remove the system from the shaking apparatus and place on the shelf of an incubator preset at 36° ± 1°C.
11 Examine the system on the incubator shelf twice daily and if positive remove for further examination. Vigorously agitate the negative systems to resuspend the erythrocytes in the broth and return to the incubator shelf. A total incubation period of at least 7 days is recommended.
12 POSITIVES – mix the contents of the chamber, unscrew the green cap and aseptically remove a sample of blood/broth mixture for subculture, microscopy and susceptibility testing. The vent in the cap contains a 0.2 micron hydrophobic membrane which ensures that the chamber is not under pressure. After sampling replace the cap on the chamber.

A POSITIVE BLOOD CULTURE, INDICATING GROWTH OF MICRO-ORGANISMS IS RECOGNISED BY THE APPEARANCE OF THE BLOOD/BROTH MIXTURE IN THE TRANSPARENT GROWTH INDICATOR DEVICE ABOVE THE LEVEL OF THE GREEN LOCKING SLEEVE.

Quality Assurance
The following organisms are used by Oxoid as part of the quality assurance of the product. The total inoculum challenge for each test organism per bottle is 10 to 50 colony forming units (CFU's).

	NTCC No.	ATCC No.
Bacillus cereus	7464	10876
Bacteroides fragilis	9343	25285
Clostridium novyi		27606
Escherichia coli	10418	10536
Fusobacterium nucleatum	10562	10953
Haemophilus influenzae	4560	19418
Klebsiella pneumoniae	11228	29665
Neisseria meningitidis	10025	13077
Peptostreptococcus anaerobius	11460	27337
Pseudomonas aeruginosa	10662	25668
Staphylococcus aureus	6571	9144
Staphylococcus epidermidis		14990
Streptococcus pneumoniae		6303
Streptococcus mutans	10449	25175
Candida albicans	(NCPF3179)	10231

User Quality Assurance
1 Examine the bottles of broth for turbidity and/or change of colour before adding any blood. Discard any bottles showing abnormal characteristics.
2 If further user quality control is required, it is recommended that 3 aerobes and 1 anaerobe from the above list be used.

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OXOID and OXOID SIGNAL are trademarks.

ISOLATOR* 1.5 TUBES

Intended use
The ISOLATOR 1.5 Tube is intended for the collection of small volume, paediatric blood samples to be used for isolation of micro-organisms. The blood sample is transferred from the tube directly to conventional agar growth media for the purpose of isolation and identification of micro-organisms.

Principles of the test
The ISOLATOR 1.5 Tube contains agents which lyse leucocytes and erythrocytes in blood, and block coagulation.

The specific agents used in the tube are:

Purified Saponin, an effective and rapid cell lysing agent, non-toxic to micro-organisms.

Polypropylene Glycol to block the foaming tendency of Saponin.

Sodium Polyanetholsulphonate (SPS) which acts as an anticoagulant, neutralises the bactericidal properties of blood and inhibits phagocytosis.

Reagents
Each ISOLATOR 1.5 Tube contains the following reagents in aqueous solution (content prior to sterilisation).

Polypropylene Glycol	8 millimetres/litre
Sodium Polyanetholsulphonate	9.6 grams/litre
Purified Saponin	40 grams/litre

The internal components of the tube are sterile.

Precautions
Used tubes, syringes and needles contain human body fluids. All materials should be handled as if they are capable of transmitting disease. Handle with appropriate care. Autoclave all used materials before discarding.

Tube reagents can cause transient eye irritation. In the event of contact with the eyes, flush with copious amounts of water and seek medical advice.

Care must be exercised to avoid injury when needles are used.

The ISOLATOR 1.5 Tube is not intended for the transportation of specimens through the mail.

FOR IN VITRO DIAGNOSTIC USE ONLY

Storage Instructions
The tubes can be stored between 2°C and 40°C; room temperature (25°C) is recommended. Turbidity within

the solution in the tube is normal. Tubes in use should be at 20°C to 30°C to ensure proper mixing of the blood with the reagents at the time of collection.

Specimen Collection and Preparation

1 Open a needle cartridge. Twist to break the tamper-evident seal. Remove cap, exposing the rear end of the needle and threaded hub. Do not remove front needle cover.

2 Assemble needle and holder. Thread needle into holder until firmly seated. Take care not to touch the needle valve to the holder.

3 Use an appropriate disinfectant (e.g. 10% PVP iodine solution) for disinfecting the stopper of the ISOLATOR 1.5 Tube. Do not allow the iodine solution to pool on the stopper. Pooling could result in the introduction of disinfectant into the tube; this may interfere with the recovery of micro-organisms.

4 Allow the disinfectant to dry completely. Insert the stopper of the ISOLATOR 1.5 Tube into the holder. Advance the tube straight onto the needle but no further than the guideline on the holder.

Blood Drawing Procedure

1 Apply a tourniquet and select a venipuncture site. Loosen the tourniquet, double cleanse and disinfect the site with an appropriate agent (e.g. alcohol and PVP iodine). Allow the disinfectant to dry for at least one minute. Always collect the ISOLATOR specimen before collecting the other specimens to avoid contaminating the blood culture.

2 Reapply the tourniquet. Remove the needle cover. Perform venipuncture with the patient's arm or other venipuncture site in a downward position. During venipuncture hold the tube/needle assembly so that the needle is elevated relative to the bottom of the tube.

   During the collection procedure, do not permit contents of the tube to contact the stopper in order to avoid the possibility of backflow of reagents from the tube with the attendant possibility of adverse patient reaction.

   Push the evacuated tube to the end of the tube holder or until blood flow is visible. When blood flows into the tube, remove the tourniquet.

3 Immediately remove the tube when fill is complete and flow has ceased (approximately 1.5ml).

4 When sampling is completed, remove the needle/holder assembly with the last tube. Apply and hold a dry sterile compress to the venipuncture site. Elevate the arm.

5 Remove the tube from the needle/holder assembly. Immediately mix the collection tube to prevent coagulation and to initiate red blood cell lysis by gently inverting the tube four or five times. Incomplete mixing will result in blood clotting in the tube.

6 Handle and discard used needle in a suitable manner.

7 Label the specimen appropriately.

Alternative needle/syringe method for specimen collection

1 Assemble a sterile needle onto a 3ml syringe or use sterile needle/syringe combinations. Loosen but do not remove the needle shield.

2 Use 10% PVP iodine solution for disinfecting the stopper of the tube.

3 Prepare the venipuncture site as previously described.

4 Remove needle shield and perform venipuncture. Collect 1.6ml of blood.

5 Add 1.5ml of the blood to the ISOLATOR 1.5 Tube by puncturing the stopper with the needle. Do not force the blood into the tube. This may cause the top to pop off the tube.

6 After removal of the needle from the tube, immediately mix the blood with the reagents in the tube by gently inverting four or five times.

7 Replace the protective cover onto the needle and discard in a suitable manner.

Specimen Processing

1 Specimens should be processed as soon as they are received in the laboratory. Immediate processing of the ISOLATOR 1.5 Tube results in faster isolation, minimises antimicrobial effects of blood, maximises the opportunity for polymicrobial isolation and may provide valuable quantitative information.

Specimens may be held in ISOLATOR 1.5 Tubes for up to 16 hours at room temperature without adverse effect on the recovery of micro-organisms. Specimens obtained from patients on antimicrobial chemotherapy should be processed immediately. Colony counts will not reflect the colony forming units per millilitre of blood if the specimen is held in the tube for more than 4 hours.

Do not refrigerate specimens collected in ISOLATOR 1.5 Tubes. The recovery of cold-sensitive organisms such as *Neisseria gonorrhoeae* may be dramatically decreased.

2 Vigorously mix the contents of the tube. A Vortex-type mixer (highest setting for 5–10 seconds) is recommended.

3 Disinfect the stopper with an appropriate disinfectant. Allow to dry for one minute.

4 Using a 3ml syringe enter the upright tube at an angle so that the needle emerges from the bottom of the stopper between the wall of the tube and the side of the stopper. Tilt (do not invert) the tube to a horizontal position and collect the blood. Be sure to remove the blood that may have accumulated in the base of the stopper.

   Expel any air in the syringe into the tube and remove the needle/syringe. Discard the tube.

5 Divide the lysate evenly among the primary isolation media, using a maximum of 0.35ml per plate. Suggested culture media and growth conditions are shown in Table 1.

6 Keeping the lids of the plates as low as possible, position the needle over the medium (don't touch the agar with the needle).

7 Dispense up to 0.35ml of inoculum in a straight

line across the surface of the plate, avoiding the edge of the agar. Discard the needle and syringe appropriately.

8 Raising the plate cover only far enough to admit a long sterile disposable or wire loop, cross-streak the inoculum starting at the top and proceeding to the bottom of the inoculum line (do not streak to edge of the agar). Rotate the plate 90 degrees and streak parallel to the original inoculum line. Rotate plate 45 degrees and streak a third time to ensure maximum distribution of the inoculum. Do not sterilise the loop between plates.

9 After plating and streaking, either appropriately discard the disposable loop, or sterilise the inoculating loop.

10 Plates should be placed under appropriate incubation conditions as soon as possible after inoculation to optimise the isolation of fastidious and anaerobic micro-organisms.

11 Incubate aerobic plates upright for the first 24 hours, and anaerobic plates upright for the first 48 hours. Thereafter incubate all plates inverted.

12 Examine all plates daily until discarded. Even when growth appears early, the plates should be reincubated to check for a second organism which may grow later. Plates should be examined with lids in place whenever possible. If a lid must be removed due to condensation or to better visualise colonies, do not remove the lid completely; raise it only high enough to examine the area in question.

Table 1.
Suggested culture media and growth conditions.

No	Medium	Incubation conditions	Discard
1	Blood Agar	Anaerobic, 35°C–37°C	6 days
1–4	Chocolate Agar	5% CO_2, 35°C–37°C	4 days
1	Sab Dext Agar	Aerobic, 22°C–30°C	8 days

Plates should be pre-dried at least overnight at room temperature. This enhances absorption of the inoculum and reduces condensation on the plate lid.

Interpretation of Results

1 If a colony appears only within the area inoculated, it should be considered a significant positive culture regardless of genus or species. While colony counts in paediatric blood cultures are generally higher than those found in adults, it is not uncommon for the counts to be low (<10 cfu/ml) during episodes of bacteraemia associated with upper respiratory tract infections or occurring after antimicrobial therapy.

2 If colonies appear on both the inoculated area and outside the inoculated area, consider the colony within the inoculated area as a positive culture and the one outside as a contaminant.

3 If a colony appears only outside the inoculated area, it may be considered a plate contaminant.

Clinical Significance

The clinical significance of a micro-organism isolated from a patient's blood should be determined by the Physician, taking into consideration the patient's history, clinical status, repetitive cultures and other pertinent laboratory findings.

ISOLATOR* 10 TUBES

Intended use

The ISOLATOR 10 Tube is intended for the collection and concentration of micro-organisms from blood and other body fluids. The tube is used by clinical laboratories to concentrate micro-organisms before transfer to conventional agar media for isolation and identification.

Principles of the test

The ISOLATOR 10 Tube contains agents which lyse leucocytes and erythrocytes in blood, and block coagulation.

The specific agents used in the tube are:

Purified Saponin, an effective and rapid cell lysing agent, non-toxic to micro-organisms.

Polypropylene Glycol to block the foaming tendency of Saponin.

Sodium Polyanetholsulphonate (SPS) which acts as an anticoagulant, neutralises the bactericidal properties of blood and inhibits phagocytosis.

Reagents

Each ISOLATOR 10 Tube contains the following reagents in aqueous solution (content prior to sterilisation).

Polypropylene Glycol	8 millimetres/litre
Sodium Polyanetholsulphonate	15.3 grams/litre
Purified Saponin	28 grams/litre

The internal components of the tube are sterile.

Precautions

Used tubes, syringes and needles contain human body fluids. All materials should be handled as if they are capable of transmitting disease. Handle with appropriate care. Autoclave all used materials before discarding.

Tube reagents can cause transient eye irritation. In the event of contact with the eyes, flush with copious amounts of water and seek medical advice.

Care must be exercised to avoid injury when needles are used.

The ISOLATOR 10 Tube is not intended for the transportation of specimens through the mail.

FOR IN VITRO DIAGNOSTIC USE ONLY

Storage Instructions

The tubes can be stored between 4°C and 40°C; room temperature (25°C) is recommended. Turbidity within the solution in the tube is normal. Tubes in use should be at 20°C to 30°C to ensure proper mixing of the blood with the reagents at the time of collection.

Specimen Collection and Preparation

1 Open a needle cartridge. Twist to break the tamper-evident seal. Remove cap, exposing the rear end of the needle and threaded hub. Do not remove front needle cover.

2 Assemble needle and holder. Thread needle into holder until firmly seated. Take care not to touch the needle valve to the holder.

3 Use an appropriate disinfectant (e.g. 10% PVP iodine solution) for disinfecting the stopper of the ISOLATOR 10 Tube. Do not allow the iodine solution to pool on the stopper. Pooling could result in the introduction of disinfectant into the tube; this may interfere with the recovery of micro-organisms.

4 Allow the disinfectant to dry completely. Insert the stopper of the ISOLATOR 10 Tube into the holder. Advance the tube straight onto the needle but no further than the guideline on the holder.

Blood Drawing Procedure

1 Apply a tourniquet and select a venipuncture site. Loosen the tourniquet, double cleanse and disinfect the site with an appropriate agent (e.g. alcohol and PVP iodine). Allow the disinfectant to dry for at least one minute. Always collect the ISOLATOR specimen before collecting the other specimens to avoid contaminating the blood culture.

2 Reapply the tourniquet. Remove the needle cover. Perform venipuncture with the patient's arm or other venipuncture site in a downward position. During venipuncture hold the tube/needle assembly so that the needle is elevated relative to the bottom of the tube.

   During the collection procedure, do not permit contents of the tube to contact the stopper in order to avoid the possibility of backflow of reagents from the tube with the attendant possibility of adverse patient reaction.

   Push the evacuated tube to the end of the tube holder or until blood flow is visible. When blood flows into the tube, remove the tourniquet.

3 Immediately remove the tube when fill is complete and flow has ceased (approximately 10ml).

4 When sampling is completed, remove the needle/holder assembly with the last tube. Apply and hold a dry sterile compress to the venipuncture site. Elevate the arm.

5 Remove the tube from the needle/holder assembly. Immediately mix the collection tube to prevent coagulation and to initiate red blood cell lysis by gently inverting the tube four or five times. Incomplete mixing will result in blood clotting in the tube.

6 Handle and discard used needle in a suitable manner.

7 Label the specimen appropriately.

Alternative needle/syringe method for specimen collection

1 Assemble a sterile needle onto a 20ml syringe or use sterile needle/syringe combinations. Loosen but do not remove the needle shield.

2 Use 10% PVP iodine solution for disinfecting the stopper of the tube.

3 Prepare the venipuncture site as previously described.

4 Remove needle shield and perform venipuncture. Collect 11ml of blood.

5 Add 10ml of the blood to the ISOLATOR 10 Tube by puncturing the stopper with the needle. Do not force the blood into the tube. This may cause the top to pop off the tube.

6 After removal of the needle from the tube, immediately mix the the blood with the reagents in the tube by gently inverting four or five times.

7 Replace the protective cover onto the needle and discard in a suitable manner.

Specimen Processing

1 Specimens should be processed as soon as they are received in the laboratory. Immediate processing of the ISOLATOR 10 Tube results in faster isolation, minimises antimicrobial effects of blood, maximises the opportunity for polymicrobial isolation and may provide valuable quantitative information.

Specimens may be held in ISOLATOR 10 Tubes for up to 16 hours at room temperature without adverse effect on the recovery of micro-organisms. Specimens obtained from patients on antimicrobial chemotherapy should be processed immediately. Colony counts will not reflect the colony forming units per millilitre of blood if the specimen is held in the tube for more than 4 hours.

Do not refrigerate specimens collected in ISOLATOR 10 Tubes. The recovery of cold-sensitive organisms such as *Neisseria gonorrhoeae* may be dramatically decreased.

2 Place the tube into an adaptor in a fixed angle rotor (35 degree) in a suitable centrifuge. Use only the unique adaptors intended for the ISOLATOR 10 Tubes. Centrifuge at 3000xg for 30 minutes. The centrifuge brake should not be used; this could disturb the concentrate and decrease recovery of micro-organisms. Be careful when removing the tube from the centrifuge to avoid mixing the supernatant fluid and the microbial concentrate.

3 The proper orientation of the adaptor is critical for the centrifugation of ISOLATOR 10 Tubes without breakage or leakage. Putting the adaptor in upside down or spinning it empty may cause it to deform. It is therefore important to properly orientate the adaptor in the rotor and to remove adaptors that are not being used before centrifugation. Periodic application of a light lubricant inside the adaptor will make tube insertion and removal easier.

4 Following centrifugation, carefully remove each ISOLATOR 10 Tube from its adaptor and place in the ISOSTAT rack. A slight clockwise twist will facilitate insertion of the tube into the rack. Be sure that tubes are firmly seated and vertically aligned, to avoid breakage while applying the cap.

5 Disinfect the stopper with an appropriate disinfectant. Do not allow the disinfectant to pool in the stopper cavity. Allow to dry for one minute.

6 Place the rack on the base of the ISOSTAT Press.

7 Remove an ISOSTAT Cap by pushing the base of the cap out through the sterile pack. To avoid contamination, handle by the sides only. Do not touch the top of the cap or the tip of the internal spike.

   Place a cap over the stopper of each ISOLATOR 10 Tube. If more than one tube is being processed,

position caps on all tubes in the rack before proceeding to the next step.

8 Position a tube with its cap under the press head. Gently pull the handle of the press down as far as possible and hold down for five seconds. The spike will penetrate the stopper and the cap will be firmly seated on top of the tube. Return the handle to the upright position.

If more than one tube is being processed, rotate the rack to position the next tube. Press the cap onto this tube, and continue until caps have been pressed onto each tube in the rack. Carefully move the rack of tubes from the press to the work area.

9 Open the heat seal at the top of a pack of ISOSTAT supernatant pipettes, then pull apart the zippered seal. Remove a supernatant pipette from the pack. To avoid contamination, handle pipettes by the bulb only. Do not touch the pipette stem.

The pack may be reclosed by pressing the edges of the zippered seal together.

10 Squeeze the bulb of the ISOSTAT supernatant pipette to collapse it and to provide a vacuum for supernatant withdrawal. Do this before inserting the stem of the pipette into the ISOLATOR 10 Tube.

Do not squeeze the pipette bulb after insertion of the pipette stem into the tube. Bubbling may disturb the microbial concentrate and result in the decreased recovery of micro-organisms. If this occurs the ISOLATOR 10 Tube should be centrifuged again. The ISOSTAT cap must be removed prior to centrifugation.

11 Carefully insert the stem of the supernatant pipette into the ISOLATOR 10 Tube through the membrane of the ISOSTAT cap while maintaining pressure on the bulb.

Insert the pipette into the tube as far as possible; the base of the bulb must rest on the cap.

Release the bulb and allow the supernatant fluid to be drawn into the pipette. Repeat this procedure with the remaining tubes in the rack, using a new pipette for each tube. Confirm that air has entered the pipettes indicating that all the supernatant fluid has been withdrawn.

12 When the supernatant fluid has been withdrawn from all ISOLATOR 10 Tubes, remove and discard the pipettes into an appropriate receptacle for contaminated waste.

13 Open the heat seal at the top of the pack of ISOSTAT concentrate pipettes, then pull apart the zippered seal. Remove a concentrate pipette from the pack.

To avoid contamination, handle pipettes by the bulb only. Do not touch the pipette stem. The pack may be reclosed by pressing the edges of the zippered seal together.

14 Remove the first ISOLATOR 10 Tube from the rack and vigorously mix the contents for 5–10 seconds in order to achieve a homogeneous emulsion. A vortex-type mixer (highest setting) is recommended.

15 Squeeze the bulb of the concentrate pipette to collapse it and to provide a vacuum for concentrate withdrawal. Do this before inserting the stem of the pipette into the tube.

Carefully insert the stem of the concentrate pipette into the ISOLATOR 10 Tube through the membrane in the ISOSTAT cap while maintaining pressure on the bulb.

Insert the pipette into the tube so that the tip reaches the bottom. It may be necessary to manipulate both pipette and tube to properly orientate the pipette tip.

Gradually release pressure on the bulb and allow the concentrate to be drawn into the pipette. A slow controlled release of the bulb is necessary to achieve maximum recovery of concentrate.

16 Immediately remove the pipette and use it to distribute the concentrate evenly onto the selected agar media. Keeping the lids of the plates as low as possible, dispense the concentrate in a straight line across the surface of the agar. Keep the inoculum away from the edge of the plate. For suggested culture media and growth conditions see Table 2.

17 Using the tip of the concentrate pipette, streak through the concentrate, making about 15 to 20 passes perpendicular to the original inoculum line. Streak lines should be kept away from the edges of the plate.

18 Discard used pipettes and ISOLATOR 10 Tubes into an appropriate receptacle for contaminated waste.

19 Plates should be placed under appropriate incubation conditions as soon as possible after inoculation to optimise the isolation of fastidious and anaerobic micro-organisms.

20 Incubate aerobic plates upright for the first 24 hours, and anaerobic plates upright for the first 48 hours. Thereafter incubate all plates inverted.

21 Examine all plates daily until discarded. Even when growth appears early, the plates should be reincubated to check for a second organism which may grow later. Plates should be examined with lids in place whenever possible. If a lid must be removed due to condensation or to better visualise colonies, do not remove the lid completely; raise it only high enough to examine the area in question.

Table 1.
Suggested culture media and growth conditions.

No	Medium	Incubation conditions	Discard
1	Blood Agar	Anaerobic, 35°C–37°C	6 days
2	Chocolate Agar	5% CO_2, 35°C–37°C	4 days
1	Sab Dext Agar	Aerobic, 22°C–30°C	8 days

Plates should be pre-dried at least overnight at room temperature. This enhances absorption of the inoculum and reduces condensation on the plate lid.

Interpretation of Results

1 If a colony appears only within the area inoculated, it should be considered a significant positive culture regardless of genus or species. In adults, bacteraemia at a level of one colony forming unit or less per millilitre of blood is common. Thereafter

the recovery of a single colony on the streak with the ISOLATOR system can be significant.

2 If colonies appear on both the inoculated area and outside the inoculated area, consider the colony within the inoculated area as a positive culture and the one outside as a contaminant.

3 If a colony appears only outside the inoculated area, it may be considered a plate contaminant.

Clinical Significance

The clinical significance of a micro-organism isolated from a patient's blood should be determined by the physician, taking into consideration the patient's history, clinical status, repetitive cultures and other pertinent laboratory findings.

* Isolator is a trademark of Carter-Wallace, Inc., New York, N.Y. 10105 USA

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DIAGNOSTIC KITS AND REAGENTS, RAPID FOOD TESTS

November 1998

DIAGNOSTIC KITS AND REAGENTS, RAPID FOOD TESTS

INTRODUCTION

Oxoid diagnostic kits are used for the identification of bacteria growing on culture media and for the direct detection of specific antibody produced due to microbial infection.

All of these products are based on particle agglutination with simple visual reading. The tests can be performed easily on single or batched samples and the provision of appropriate controls ensures the reliability of results.

The tests are rapid and offer the benefit of minimal sample preparation with actual assay times of between only 20 seconds for the Staphytect kit up to eight minutes for the VDRL kit.

Three particle types are used for the assays; stabilised sheep red blood cells, carbon particles and blue coloured latex particles. All of these formats give highly visible reactions when viewed against the supplied, high quality white disposable reaction cards. Most of the tests are based upon antibody/ antigen reactions, which are made visible by the presence of the coloured particles.

Culture Confirmation Tests for the Identification of Plate Isolates
As an example, the test reagent in the *E. coli* 0157 kit (DR620) consists of a suspension of sub-micron blue latex particles which have been sensitised with rabbit antibodies that react specifically with the lipopolysaccharide found on *E. coli* strains of the serotype 0157. When a portion of a colony of *E. coli* 0157 is emulsified with the latex reagent, the antibody present on the latex surface binds the LPS on the cells and also any antigen fragments present. This binding causes considerable cross-linking of the latex particles to produce large clumps. The clumps, when of a sufficient size become visible to the naked eye during the rocking of the card. In addition to the presence of the clumps the latex particles are removed from the liquid phase causing a noticeable (and often complete) clearing of the smooth blue background. In a negative reaction (when the organism does not carry the 0157 antigen) the latex particles do not bind to the bacteria and therefore no cross-linking occurs and the blue suspension remains smooth.

Other culture confirmation test kits currently available are listed below.

Direct Tests for Detection of Specific Antibody
The *Helicobacter pylori* latex kit (DR700) is an example of a test that may be used to test a specimen directly. It utilises antigen-sensitised blue latex particles to detect circulating antibodies present in patients who are infected with the *H. pylori* bacterium. Specific antibodies if present will cross-link the latex particles causing visible agglutination.

Other direct test kits currently available are listed below.

All Oxoid diagnostic kits feature ready to use reagents, clear multi-language labelling and instructions and a work station format for ease of use and storage. The disposable test cards are interchangeable within all of the kits except the VDRL kit and all card types are available separately if required. Individual reagents are also available, where stated to enable replenishment of kits or for more limited testing.

New products are regularly being introduced into the Diagnostic Reagent range to cover an ever wider range of analytes.

CAMPYLOBACTER TEST KIT

Code: DR150

The Oxoid Campylobacter Test Kit is a latex agglutination test for the identification of enteropathogenic Campylobacter spp. from solid culture media.

Introduction
Campylobacters are helical, or curved, Gram-negative, oxidase positive rod-shaped bacteria.[1] They have been isolated from the environment as well as from humans and animals. The adoption of Campylobacter culture in laboratory routines for investigating enteritis has shown *Campylobacter* spp. to be the leading cause of diarrhoeal disease. Infection has been associated with the consumption of contaminated water and foods, particularly poultry and unpasteurised milk.

Current methods for the isolation and culture of Campylobacters have been recently reviewed.[2]

The Dryspot Campylobacter test reagent consists of blue latex particles sensitised with rabbit antibody reactive with selected Campylobacter cell surface antigens. The control reagent consists of blue latex particles sensitised with rabbit antibody not reactive with Campylobacters.

The latex reagents are dried onto reaction cards. When a Campylobacter extract is mixed with the test reagent, agglutination occurs due to cross-linking of latex-bound antibody and Campylobacter antigens. If the extract does not contain recognised Campylobacter antigens agglutination will not occur and the result will be negative.

The Oxoid Dryspot Campylobacter Test includes antigen extraction reagents and a positive control antigen preparation.

Components of the Kit
DR151M Dryspot Campylobacter Reagent Cards

Test areas: Blue latex particles sensitised with rabbit antibody reactive with selected Campylobacter cell surface antigens.

Control areas: Blue latex particles sensitised with rabbit antibody not reactive with Campylobacters.

10 foil-sealed plastic trays each containing five reaction cards and a desiccant pouch. Each card has a test and a control area. 50 tests in total.

DR152M Extraction Reagent 1
A solution of acetic acid (1.2m)

DR153M Extraction Reagent 2
A neutralising reagent of Tris buffer containing 0.09% sodium azide as a preservative.

DR154M Positive Control Reagent
Consists of a neutralised acid extract of appropriate Campylobacter organisms in buffer containing 0.09% sodium azide as a preservative.

DR699M Paddle Pastettes

DR155M Storage Bag

Instructions for use.

Materials Required but not Provided
Timer

Sterile Loop (5μl calibrated)

12 x 75mm test tubes

A suitable laboratory disinfectant.

For full procedure please see product insert.

References

1 Cowan S. T. and Steel K. J. (1965) Characters of Gram-negative bacteria. In *Manual for the identification of medical bacteria.* Barrow G. I. and Feltham R. K. A. (ed.) Third Edition. Cambridge University Press. Cambridge, U.K.

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E. COLI 0157 LATEX TEST

Code: DR620

A latex agglutination test for the identification of E. coli Serogroup 0157.

Certain strains of *Escherichia coli* have recently been implicated in some cases of haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS).

It has been shown that these strains produce a vero-cytotoxin (VT). The *E. coli* serotype most frequently isolated from HC and HUS cases is 0157:H7. Isolation of this serotype from a diarrhoeal stool, especially with blood, is indicative of a verocytotoxin-producing strain[1,2,3,4,5,6,7,8].

The Oxoid *E. coli* 0157 Latex Test will demonstrate by slide agglutination *E. coli* strains possessing the 0157 antigen. The test is best used in conjunction with Sorbitol MacConkey Agar (Oxoid CM813). *E. coli* 0157:H7 strains do not ferment sorbitol and therefore give colourless colonies on this medium. The majority of *E. coli* isolates do ferment sorbitol and give characteristic pink colonies.

Sorbitol MacConkey Agar should be used as the primary screen. Non-sorbitol-fermenting colonies can then be tested with the latex reagents, to determine if the isolate belongs to the 0157 serogroup and therefore a potential VT-producing strain.

References

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2 Konowalchuk J., Speirs J. and Stavric S. (1977) *Infect. Immune 18. 775–779.*

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INFECTIOUS MONONUCLEOSIS KIT

Code: DR680

The Oxoid Infectious Mononucleosis Kit is a simple, two-minute latex agglutination test for the detection of the heterophile antibody associated with infectious mononucleosis in serum and plasma.

Infectious mononucleosis (glandular fever) is an acute infectious disease caused by the Epstein-Barr virus and primarily affects lymphoid tissue. It is characterised by the appearance of enlarged and often tender lymph nodes, enlarged spleen, and abnormal lymphocytes in blood. Patients usually, but not always, develop a transient heterophile antibody response.

The detection of heterophile antibodies to infectious mononucleosis by the agglutination of sheep red blood cells was first reported by Paul and Bunnell[1]. Subsequent work by Davidsohn,[2,3] Lee[3] and Beer[4] showed the need for differential absorption of sera to remove non-infectious mononucleosis heterophile antibodies. Fletcher and Woolfolk[5] showed that antigens obtained from the membranes of bovine erythrocytes were more effective in combining with the infectious mononucleosis heterophile antibodies than those antigens obtained from either sheep or horse erythrocytes[6,7].

References

1 Paul J. R. and Bunnell W. N. The presence of heterophile antibodies in infectious mononucleosis. *Am. J. Med. Sci. 1932; 183. 90–104.*

2 Davidsohn I. Serologic diagnosis of infectious mononucleosis. *JAMA 1937: 108. 289–295.*

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6 Data on file at Oxoid Limited.

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Paddle Pastettes is a registered trademark of Alpha Laboratories.

LEGIONELLA LATEX TEST

Code: DR800

The Oxoid Legionella Latex Test is a latex agglutination test for the identification of predominant *Legionella* species grown on plate media from patients with suspected *Legionellosis* or from environmental sources. The Oxoid Legionella Latex Test allows separate identification of *Legionella pneumophila* serogroup 1 and serogroups 2–14 and detection of seven other *Legionella* species which have been implicated in human disease.

Introduction
Legionnaires' disease named after the outbreak in 1976 at the American Legion Convention in Philadelphia, is caused by *Legionella pneumophila* and other *Legionella* species. It is characterised as an acute febrile respiratory illness ranging in severity from mild illness to fatal pneumonia. Since that time, it has been recognised that the disease occurs in both epidemic and endemic form and that the sporadic cases are not readily differentiated from other respiratory infections by clinical symptons. It is estimated that worldwide about 25,000 cases of Legionella infections occur annually. Known risk factors include immunosuppression, cigarette smoking, alcohol consumption and concomitant pulmonary disease. The mortality rate, which can be as high as 25% in untreated immunosuppressed patients, can be lowered if the disease is diagnosed rapidly and appropriate antimicrobial therapy started earlier.

Legionella pneumophila has been shown to be a major cause of both pneumonia and an acute self limiting febrile disease called Pontiac Fever. *L. pneumophila* strains and other *Legionella* species are isolated from patients with pneumonia and from the environment (mainly water).

Rare isolations have also been made in cases other than pneumonia, such as wound abscesses. The major reservoir of *Legionella* species appears to be fresh water sites, air-conditioning units and various water plumbing fixtures.

L. pneumophila is the most common cause of Legionnaires' disease. At present, 14 different serotypes exist of which *L. pneumophila* serogroup 1 accounts for 90% of cases.

The Oxoid Legionella Latex Test uses antibody sensitised blue latex particles which will agglutinate in the presence of specific Legionella cell wall antigens to form visible clumps. This provides a fast and simple screening procedure for predominant pathogenic *Legionella* species and serotypes[1,2].

Components of the Kit
DR801 *Legionella pneumophila* serogroup 1 Test Reagent
Consists of blue latex particles sensitised with specific rabbit antibody reactive with *Legionella pneumophila* serogroup 1 antigen. Each kit contains sufficient reagent for 50 tests.

DR802 *Legionella pneumophila* serogroup 2–14 Test Reagent
Consists of blue latex particles sensitised with specific rabbit antibody reactive with *Legionella pneumophila* serogroup 2–14 antigen. Each kit contains sufficient reagent for 50 tests.

DR803 *Legionella* species Test Reagent
Consists of blue latex particles sensitised with specific rabbit antibody reactive with the following species and serotypes:
L. longbeachae 1 & 2
L. bozemanii 1 & 2
L. dumoffii
L. gormanii
L. jordanis
L. micdadei
L. anisa

Each kit contains sufficient reagent for 50 tests.

DR804 Positive Control Suspension
A polyvalent suspension of Legionella cells in buffer, sufficient for 25 tests.

DR805 Negative Control Suspension
A suspension of *L. spiritensis* cells in buffer non-reactive with the test reagents sufficient for 25 tests.

DR806 Control Latex
Consists of blue latex particles sensitised with non-reactive rabbit globulin. Each kit contains sufficient reagent for 50 tests.

DR807 Suspension Buffer
A phosphate buffered saline solution. pH 7.3.

DR500 Reaction Cards
There are 50 disposable reaction cards provided in the kit.

Instruction leaflet

Materials Required but not Provided:
Microbiological loop and bunsen burner.

0.85% saline (for optional tube method).

Suitable laboratory disinfectant e.g. Sodium hypochlorite solution > 1.3% w/v.

For full procedure please see product insert.

References

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7 Data on file Oxoid Ltd.

OXOID-PYR

Code: DR580

Oxoid-PYR is a rapid colorimetric test for the determination of PYRase activity in streptococcal organisms.

Introduction
The Oxoid-PYR test for the detection of PYRase in streptococcal organisms offers a rapid diagnostic alternative to time-consuming culture methods such as growth in 6.5% NaCl. PYRase activity is indicative of enterococci or Group A streptococci. In association with serological grouping this test will provide identification of enterococci or Group A streptococci. All enterococci are PYRase positive. Group D streptococci (*Streptococcus bovis*, previously called non-enterococci) are negative.

The Oxoid-PYR test utilises test cards impregnated with substrate for the detection of PYRase activity. The enzymatic hydrolysis of this substrate by enterococci or Group A streptococci produces a red colour upon the addition of the colour developer.

Components of the Kit
Each Oxoid-PYR kit contains the following reagents sufficient for 50 tests:

Test cards
50 test cards each containing filter paper impregnated with L-pyroglutamic acid.

Buffer
1 dropper bottle containing 28ml of saline solution with 0.1% sodium azide.

Colour Developer
1 dropper bottle containing 14ml of P-Dimethylaminocinnamaldehyde.

Materials Required but not Provided:
Wooden mixing sticks or microbiological loop.

For full procedure please see product insert.

References

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STAPHYTECT

Code: DR650

Staphytect is a latex slide agglutination test[1] for the differentiation of staphylococci which possess Clumping factor and/or Protein A from those that do not.

Principle of the Test
Traditionally differentiation between coagulase positive and negative staphylococci has been performed with the tube coagulase test that detects extracellular staphylocoagulase or the slide coagulase test that detects the clumping factor (bound coagulase) present on the bacterial cell surface. Several other differentation tests are also available including the passive haemagglutination test (Oxoid Staphylase) and the DNase test.

It has been reported that approximately 97% of human strains of *Staphylococcus aureus* possess both bound coagulase and extracellular staphylocoagulase.

Protein A is found on the cell surface of about 95% of human strains of *S. aureus* and has the ability to bind the Fc portion of immunoglobulin G (IgG)[2].

The Staphytect test consists of blue latex particles coated with both human fibrinogen and IgG. On mixing the latex reagent with colonies of staphylococci which have clumping factor or Protein A present on the bacterial cell surface cross linking will occur giving visible agglutination of the latex particles. Such agglutination will occur notably with *S. aureus*. Agglutination may also occur with other species which possess clumping factor or Protein A such as *Staphylococcus hyicus* and *Staphylococcus intermedius.* If neither clumping factor nor Protein A are present no agglutination will occur and the result will be regarded as negative. The most frequent coagulase and Protein A negative isolates of staphylococci are *Staphylococcus epidermidis.*

Components of the Kit
DR651 Staphytect Test Reagent
Blue latex particles coated with both human fibrinogen and IgG. The human plasma proteins used in the manufacture of the reagent have been tested for the presence of the antibody HIV (Human Immunodeficiency Virus) and HBsAg (Hepatitis B surface antigen) and found to be non-reactive.

DR652 Staphytect Control Reagent
Blue unsensitised latex particles.

DR500 Reaction cards

Disposable reaction cards are provided in each kit. Each card may be used for 6 tests. If fewer tests are performed the card may be cut with scissors and the unused rings saved for later use.

Instruction Leaflet

Materials Required but not Provided:

Timer

Microbiological loop and bunsen burner

Disinfectant

For full procedure please see product insert.

References

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2 Taussig M.J. (1984) *Processes in Pathology and Microbiology 2nd Ed. 520–530.*

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STAPHYLASE TEST

Code: DR595

A rapid slide identification test for Staphylococcus aureus.

The generally accepted identifying characteristic of *Staphylococcus aureus* is the ability to produce coagulase. The presense of this coagulase (or 'clumping factor') may be detected in a number of ways. The Oxoid Staphylase Test detects the presence of coagulase through clumping of fibrinogen-sensitised sheep red blood cells.[1,2] The specificity of the reaction is ensured by a simultaneous test with a control reagent (unsensitised sheep red blood cells), when of course no clumping reaction should be observed.

References

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STREPTOCOCCAL GROUPING KIT

Code: DR585

A latex agglutination test for the identification of streptococcal groups A, B, C, D, F, and G.

Lancefield[1] showed that the majority of pathogenic streptococci possess specific carbohydrate antigens, which permit the classification of streptococci into groups. These streptococcal group antigens can be extracted from the cells and their presence demonstrated with latex particles previously coated with group-specific antibodies. These latex particles will agglutinate in the presence of homologous antigen, but will remain in smooth suspension in the absence of such antigen. The Oxoid Streptococcal Grouping Kit is such a latex agglutination test for the identification of the streptococcal group, and reagents are provided for groups A, B, C, D, F and G. The use of a new enzymatic extraction procedure considerably shortens the time required for antigen extraction and much improves the antigen yield, particularly for Group D streptococci[2,3,4,5,6,7].

British patent application 8414273.

References

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4 Birch B. R., Keaney M. G. L. and Ganguli L. A. (1984) *Lancet i 856–857.*

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6 Kloos W. E., and Jorgensen J. H. (1985) in *'Manual of Clinical Microbiology' 4th Edition, pp.143–153.*

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STREPTOCOCCAL GROUPING KIT ANTIGEN EXTRACTION BY NITROUS ACID

Code: DR575

This kit is a streptococcal grouping test utilising nitrous acid extraction for the rapid identification of β haemolytic streptococci of Lancefield types A, B, C, F & G.

Introduction

β-haemolytic streptococci can be differentiated into Lancefield groups based on specific carbohydrate antigens[1]. Differentiation is necessary for clinical treatment and for epidemiological purposes[2]. For extraction of the group specific antigen prior to grouping a variety of methods have been used including hot acid[1], hot formamide[3] and enzyme extraction methods[4,5]. The Oxoid Streptococcal Grouping Kit (DR585) utilises an enzyme extraction technique. This requires a 10 minute incubation period and efficiently extracts streptococci of Lancefield Groups A, B, C, D, F and G[6]. The Oxoid Nitrous Acid Kit is based on modified nitrous

reagents[6,7], which will rapidly extract the group antigens without the requirement for any incubation. However, the group D antigen is not extracted as efficiently and therefore an alternative method must be used to confirm suspected group D streptococci.

Procedure
The test is intended for use with streptococci colonies that are β-haemolytic on blood agar. The group specific antigens are extracted from streptococci by using an instant room temperature nitrous acid extraction procedure. The extract is then neutralised and the antigens are identified by agglutination.

Components of the Kit
DR576 Extraction Reagent 1
One bottle containing 8ml of sodium nitrate solution with a pH indicator. The reagent contains 0.095% sodium azide.

DR577 Extraction Reagent 2
One bottle containing 8ml of 0.4N hydrochloric acid.

DR578 Extraction Reagent 3
One bottle containing 8ml of a neutralising solution with 0.095% sodium azide.

DR586 Latex Grouping Reagent A

DR587 Latex Grouping Reagent B

DR588 Latex Grouping Reagent C

DR590 Latex Grouping Reagent F

DR591 Latex Grouping Reagent G

DR592 Polyvalent Positive Control

DR500 Disposable Reaction Cards

Instruction Leaflet

Material Required but not Provided in the kit:
Microbiological loop

12 x 75mm test tubes

Pasteur pipette

Suitable laboratory disinfectant

Precautions
This product is for *in vitro* diagnostic use only.
DO NOT FREEZE.

Reagents contain 0.095% sodium azide as a preservative. Sodium axide is toxic and may react with lead or copper plumbing to produce metal azides which are explosive by contact detonation. To prevent azide accumulation in plumbing flush with copious amounts of water immediately after waste disposal.

Specimen materials may contain pathogenic organisms, handle with the appropriate precautions. The extraction procedure may not kill bacteria therefore the extract must be handled with the same precautions.

Extraction Reagents 1, 2 contain a mild irritant and a weak acid respectively. Avoid direct contact by wearing suitable protective equipment. If the material comes into contact with the skin, mucous membranes or eyes immediately wash the area by rinsing with plenty of water.

Storage
This kit must be stored 2–8°C. Under these conditions the reagents will retain their reactivity until the expiry date shown on the kit box.

For full procedure please see product insert.

References

1 Lancefield R.C. (1938) *Proc. Soc.Exp. Biol. 38: 473–478.*
2 Facklam R.R. and Washington J.A. II. (1991) *Streptococcus and Related catalase-Negative Gram-Positive Cocci. p.238–257.* In A. Balows, W. Hausler, K.L. Herrmman, H.D., Isenberg and H.J. Shadomy (Ed) *Manual of Clinical Microbiology, 5th Ed. American Society for Microbiology, Washington, D.C. Pages 238–257.*
3 Fuller A.T. (1938) *Brit. J. Exp. Path. 19: 130–139.*
4 Maxted W.R. (1948) *Lancet, ii. 255–256.*
5 Ederer G.M., Herman M.M., Bruce. R., Matsen J.M. and Chapman S.S. (1972) *Appl. Microbiol. 23: 285–288.*
6 Data on File, Oxoid.
7 El Kholy A., Wannamaker L.W. and Krause R.M. (1974) *Appl.Microbiol., 28: 836–839.*
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12 Lawrence J., Yajko D.M. and Hadley W.K. 1985 *J. Clin. Microbiol. 22: 772–777.*

TPHA TEST

Code: DR530

Treponema pallidum haemagglutination test (TPHA) for the serodiagnosis of Syphilis.

Introduction
Syphilis is a sexually transmitted disease. The causative organism is *Treponema pallidum*, a spirochaete which cannot be grown on culture media or in tissue culture. Diagnosis of infection is normally by the detection of antibody specific for *Treponema pallidum* in the patient's blood or CSF.

Detection of the antibody becomes possible after 3–4 weeks following infection. Detectable levels may remain for long periods after treatment. Two groups of antibodies are formed in response to infection:

1 Antibodies reactive with non-treponemal antigens (reagin antibodies)

Reagin antibodies are normally found in the active disease. They are detected by the VDRL/Carbon Antigen and RPR tests (Oxoid VDRL Carbon Antigen Test (DR525M)). Reagin antibodies levels subside after successful treatment[1].

The non-treponemal antibodies may arise for reasons other than syphilitic infection. Positive tests for these should therefore be confirmed by a test for the specific antibodies.

2 Antibodies reactive with the specific antigens of *T. pallidum:*

Specific antibodies persist long after the infection has been successfully treated.

The TPHA test will detect these antibodies. The Oxoid TPHA test is a sensitive passive haemagglutination test specifically for the detection of antibodies to *Treponema pallidum.*

Components of the Kit (DR530)

DR531 Test Cell Suspension
2 bottles each containing 8.5ml of antigen coated formolised tanned fowl erythrocytes. The dropper bottle will dispense 75μl drops. Each kit contains sufficient suspension for 200 tests.

DR532 Control Cell Suspension
2 bottles each containing 8.5ml of uncoated formolised tanned fowl erythrocytes. The dropper bottle will dispense 75μl drops. Each kit contains sufficient suspension for 200 tests.

DR533 Diluent Buffer
2 bottles each containing 20ml of buffer.

DR534 Positive Control Serum
1 bottle containing 2ml of pre-diluted (1/20) serum, positive for antibodies to *T. pallidum*. The serum should cause agglutination in the screening test and remain positive to a serum dilution of 1/2560 plus or minus one doubling dilution in the quantitative test.

DR535 Negative Control Serum
1 bottle containing 2ml of pre-diluted (1/20) serum negative for antibodies to *T. pallidum.*

The human sera used in the manufacture of the controls have been shown to be negative for HBsAG (Hepatitis B surface antigen), Hepatitis C and HIV 1 and 2 antibodies by FDA approved tests.

Instruction Leaflet

The following materials are required but not provided.

U-well microtitration plate.

Micropipettes and tips to deliver 25 and 100μl volumes.

Suitable laboratory disinfectant.

For full procedure please see product insert.

References

1 Garner M. F., Backhouse J. L., Daskalopoulos G. and Walsh J. L. (1973) *J. Clin Path.* 26. 258–260.
2 Rathlev T. (1965) W.H.O. VDT/RES/77 65.
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10 Coffey E. M., Bradford L. L., Naritomi L. S. and Wood R. M. (1972) *Appl. Microbiol.* 24. 26–30.
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VDRL TEST KIT

Code: DR525

A test kit for the detection and quantitative assessment of reagin antibodies in syphilis screening.

The Oxoid VDRL Test Kit is a macroscopic non-treponemal flocculation test for use in the detection and quantification of reagin antibodies. A presumptive diagnosis of syphilis can be made when these antibodies are detected in serum. The Card Test uses a modified form of the VDRL antigen[1] containing micro-particulate carbon to improve the reading of results.

The test can be performed using unheated serum or plasma. The VDRL Carbon Antigen Suspension can also be used on both single and multi-channel Auto-Analyser equipment[2,3,4,5,6].

VDRL ANTIGEN SUSPENSION

Code: DR526

Oxoid VDRL Carbon Antigen Suspension is used for the detection of reagin antibodies, which indicate a serological diagnosis of syphilis. It is a modified form of VDRL antigen utilising micro-particulate carbon to enhance the visual reading of results. The product is suitable for use on both single and multi-channel Anto-Analyser equipment and for a manual slide test[2,3,4,5,6].

VDRL CARBON ANTIGEN SUSPENSION

Code: DR520

Oxoid VDRL Carbon Antigen Suspension is used for the detection of reagin antibodies, which indicate a serological diagnosis of syphilis. It is a modified form of the VDRL antigen utilising micro-particulate carbon to enhance the visual reading of results. The product is suitable for use on both single and multi-channel Auto-Analyser equipment and for a manual slide test[2,3,4,5,6].

References

1 Portnoy J., Brewer J. H. and Harris A. D. (1962) *US Public Health Report 77. 645.*
2 McGrew B. E., Stout G. W. and Falcone V. H. (1968) *Am. J. Med. Tech. 34. 634.*
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4 Norins L. C. (1968) Automation in Analytical Chemistry, *Technicon Symposium 1967, 1 157 New York Mediad.*
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DRYSPOT RANGE

In 1997 the Oxoid Dryspot range was launched. The new range consists of easy-to-use latex slide agglutination tests using dry reagent technology called Dryspot.

The Dryspot range is characterised by the use of sensitive blue latex reagents which are dried on to the surface of specially designed reaction cards or test sticks. This convenient format replaces bottles of wet reagents, eliminating the need for refrigeration and dispensing of reagents.

The reaction cards and test sticks are packed in re-sealable moisture-proof pouches which may be stored at room temperature thus easing demands on refrigerator space. A further significant benefit is that all kits have a shelf life of two years.

DRYSPOT E. COLI O157 LATEX TEST

Code: DR120M

A latex slide agglutination test for the identification of E. coli Serogroup O157.

The Oxoid Dryspot *E. coli.* O157 Latex Test will demonstrate by slide agglutination *E. coli* strains possessing the O157 antigen. The test is best used in conjunction with Sorbitol MacConkey Agar (Oxoid CM813). *E. coli* O157:H7 strains do not ferment sorbitol and therefore give colourless colonies on this medium. The majority of *E. coli* isolates do ferment sorbitol and give characteristic pink colonies.

Sorbitol MacConkey Agar should be used as the primary screen. Non-sorbitol-fermenting colonies can then be tested with the latex reagents, to determine if the isolate belongs to the O157 serogroup and therefore a potential VT-producing strain.

Contents of the Kit
Dryspot *E. coli* O157 Reagent Cards.

Blue latex particles coated with polyclonal antibodies specific to the O157 antigen of *E. coli*.

4 pouches each containing 10 cards plus a moisture absorbent sachet.

3 test and 3 control reaction areas on each card, 120 tests in total.

1 pouch containing 3 positive control strips (10 sticks – pink spots) and 3 negative control strips (10 sticks – green spots).

Plastic pouch clip for sealing of opened pouches.

Instruction leaflet.

DRYSPOT IM

Code: DR180M

A latex slide agglutination screening test for the detection of infectious mononucleosis (IM) heterophile antibody in serum or plasma.

Introduction
Dryspot IM is a simple two minute latex agglutination test for the detection of heterophile antibody associated with infectious mononucleosis (IM) in serum and plasma.

IM syndrome is caused by Epstein-Barr virus (EBV) and usually presents symptoms including fatigue, pharyngitis, malaise, fever, jaundice, lymphadenopathy and splenomegaly[1,2]. Since these symptoms are not specific for IM, serological evidence is necessary to confirm all suspected cases[3,4]. Antigens obtained from the membrane of bovine erythrocytes have been shown to be more effective than those obtained from other species[5], in detection of heterophile antibody.

In the Dryspot IM test, purified antigen from bovine red cell membranes is used to coat latex particles which are presented as dry spots on the surface of a reaction card.

Contents of the Kit
Dryspot IM test reagent cards.
Blue latex particles sensitised with purified bovine antigen.

2 pouches each containing 10 cards and a moisture absorbent sachet.

3 test reaction areas on each card, 60 tests in total.

Positive control strips (10 sticks – pink spots)

Pink-dyed rabbit antiserum containing specific antibody reactive with the test latex.

Negative control strips (10 sticks – green spots)

Green-dyed rabbit serum not reactive with the test latex.

Paddle pastettes.

Plastic pouch clip for sealing of open pouches.

Instruction leaflet

Materials required but not provided:

Timer

A suitable laboratory disinfectant.

Additional items required for optional semi-quantitative assay:

Test tube (12 x 75mm)

Pipettes (for delivery of 0.5 ml)

Saline (0.9%)

References

1 Lennette E.T. and Henle W. (1997) Clinical and Serological Features, *Lab. Management.* 25.23–28.
2 Cook L., Modget J. and Willis D. et al. (1997) *J. Clin. Microbiol.* 25(12). 2391–2394.
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DRYSPOT STAPHYTECT PLUS

Code: DR100M

A latex slide agglutination test for the differentiation of clinically important staphylococci, including MRSA.

Introduction
Dryspot Staphytect Plus is a latex slide agglutination test[1] for the differentiation of staphylococci which possess clumping factor, Protein A and certain capsular polysaccharides, found in MRSA strains, from those which do not.

It has been reported that approximately 97% of human strains of *Staphylococcus aureus* possess both bound coagulase and extracellular staphylocoagulase.

Protein A is found on the cell surface of about 95% of human strains of *S. aureus* and has the ability to bind the Fc portion of immunoglobulin G (IgG)[2].

Dryspot Staphytect Plus is designed to identify these markers by using blue latex particles coated with both porcine fibrinogen and rabbit IgG, including specific polyclonal antibodies raised against capsular polysaccharides of *S. aureus*[3.4].

Components of the Kit
DR101M Dryspot Staphytect Plus Reagent Cards

Blue latex particles coated with both porcine fibrinogen and rabbit IgG together with specific polyclonal antibodies raised against capsular polysaccharide of *S. aureus*.

4 pouches each containing 10 cards and a moisture absorbent sachet.

3 test and 3 control reaction areas on each card, 120 tests in total.

Plastic pouch clip for sealing of opened pouches

Instruction leaflet

Materials required but not provided:

Saline (0.9%)

Timer

Pipette or dropper (50µl)

Microbiological Loop

A suitable laboratory disinfectant

References

1 Essers L. and Radebold, K. (1980) *J.Clin. Microbiol. 12. 641–643.*
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DRYSPOT STREPTOCOCCAL GROUPING KIT

Code: DR400M

A grouping kit for the rapid differentiation of β-haemolytic streptococci types A, B, C, D, F and G.

Introduction
The differentiation of β-haemolytic streptococci into Lancefield types A, B, C, D, F and G can be achieved for clinical or epidemiological purposes[1] using specific carbohydrate antigens[2].

Various methods have been used to extract the group specific antigen prior to grouping. These include hot acid[2], hot formamide[3] and enzyme extraction methods[4,5].

The Dryspot Streptococcal Grouping Kit can be used with an enzyme extraction technique (Oxoid DR593G). This requires a 10 minute incubation period and efficiently extracts antigens from group A, B, C, D, F and G streptococci[6]. Alternatively, it may be used with nitrous acid reagents (Oxoid DR574M) which will instantly extract the group antigens (except for group D) without the need for incubation.

Components of the Kit
Dryspot test reagent sticks.

Blue latex particles sensitised with rabbit antibody to appropriate group specific antigen (Lancefield types A, B, C, D, F and G).

6 pouches each containing 6 strips of 10 test reagent sticks.

60 tests in total for each group.

6 positive control sticks (10 sticks – pink spots)

60 white disposable reaction cards.

Plastic pouch clip for sealing of opened pouches.

Oxoid Extraction Enzyme Reagent (DR593G).

Instruction leaflet.

Materials required but not provided:

Microbiological loop

12–35mm test tubes

Pasteur pipettes

Suitable laboratory disinfectant

Saline (0.9%)

Distilled or deionised water

Timer

References

1 Facklam R.R. and Washington J.A.H. (1991) Streptococcus and Related Catalase-Negative Gram-Positive Cocci. In *Manual of Clinical Microbiology* 5th Edition. 237–257. A. Balows, W. Hausler, K.I. Kerrman, H.D. Isenberg and H.J. Shadony (ed.). American Society for Microbiology. Washington D.C.
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6 Data on file, Oxoid.

RAPID FOOD TESTS

(AOAC and AFNOR approved)

OXOID LISTERIA RAPID TEST

Code: FT401

Intended Use
The Oxoid Listeria Rapid Test is designed for the detection of *Listeria* species in foods and environmental samples within 43 hours. The test protocol allows for the availability of the result two working days after the sample is received in the testing laboratory. The procedure uses two carefully selected enrichment steps for the maximum recovery and growth of Listeria followed by an immunoassay in the Clearview™ format. This simple system gives a clear visual result 20 minutes after the addition of the heated and cooled sample to the test device with no further manipulations being required.

Introduction
Listeria is a genus of Gram positive, non-sporing bacilli with a DNA G+C content of 36–38%. They have up to 6 peritrichous flagella and are motile when grown at 30°C or below. They are aerobic and facultatively anaerobic, catalase positive and oxidase negative. The genus comprises six species, *L. monocytogenes, L. ivanovii, L. innocua, L. welshimeri, L. seeligeri* and *L. grayi* subsp. *Grayi* and *L. Grayi* subsp. *murrayi*. All Listeria, except *L. grayi*, variously share 4 flagella antigens A, B, C, and D, of which flagella antigen B is common[1].

Pathogenic and non-pathogenic Listeria are ubiquitous in nature and can be isolated from soil, vegetables and natural waters as well as from healthy animals and man. They are able to grow over a temperature range of 1–45°C. Consequently, *L. monocytogenes* is a food poisoning risk to susceptible individuals if present in foods that are subsequently stored at these temperatures for sufficient time for the organism to grow to infectious levels before ingestion. Clinical symptoms include flu-like illness, spontaneous abortion, still birth, meningitis, pneumonitis, septicaemia and endocarditis. *Listeria monocytogenes* infections mainly occur in neonates, pregnant women, the elderly and immunocompromised individuals.

Test Principle
1 Enrichment Broth System
Culture of the test sample is in two sequential enrichments, taking 42 hours. Any Listeria organisms present in the food or environmental sample are selectively enriched using growth conditions which are optimal for flagella expression.

2 Antigen Extraction
The second enrichment media is heated at 80°C for 20 minutes to extract the flagella antigen.

3 Clearview™ Listeria Device
The Clearview Listeria device contains specific monoclonal antibodies to the B flagella antigen[2] that is common to the *Listeria* species indicated earlier.

The extracted antigen is added onto a pad in the Sample Window. This contains blue latex labelled with antibody. The extract rehydrates the complex and the specific antigen reacts, if present, with the antibody.

The complex moves through the pad by capillary action to a test strip containing an immobilised line of antibody midway along the Result Window.

A further reaction between antigen/latex complex and the fixed antibody results in a blue line in the Result Window.

If no flagella antigen is present the Result Window will remain clear.

The Clearview Listeria device also provides an integral control feature. The appearance of a blue line in the Control Window shows the test has been carried out correctly.

Components of the Kit
Oxoid SR166M Half Fraser Supplement: 50 vials
Half Fraser Supplement is used in conjunction with Fraser Broth (CM895), it is modified by the addition of only half the level of selective agents normally found in Fraser Supplement. Each vial is sufficient for 225ml of broth.

Clearview Listeria Test Units: 50

Positive Clearview Control: 3 vials
Non-viable *Listeria monocytogenes* suspension.

Instruction leaflet

Materials Required but not Provided:
Oxoid Fraser Broth, (FB) CM895

Oxoid Buffered Listeria Enrichment Broth, (B.L.E.B.) CM 897

B.L.E.B. Selective Enrichment Supplement, SR141E

Incubator at 30 ± 2°C

Water bath at 80 ± 2°C

Glass test tubes of 5–8ml capacity

Sterile water/ethanol mixture 1:1, (v/v).

For full procedure please see product insert.

References

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SALMONELLA RAPID TEST

Code: FT201

Introduction
The Oxoid Salmonella Rapid Test is for the presumptive detection of motile salmonellae in raw food materials, finished food products and factory environmental samples.

Principle
Pre-enrichment of a homogenised sample in suitable medium is followed by inoculation of a culture vessel containing a Salmonella Elective Medium and two tubes. Each tube contains a selective medium and an upper indicator medium separated by a porous partition. Salmonellae migrate actively through the lower selective media to the upper indicator media where their presence is indicated by a colour change.

Components of the Kit
50 Culture vessels

Each culture vessel contains two tubes.
Tube A (Blue Cap) contains modified Rappaport-Vassiliadis Medium as the selective medium and modified Lysine Iron Cystine Neutral Red Medium as the indicator medium.

Tube B (Red Cap) contains modified Lysine Iron Desoxycholate Medium as the selective medium and modified Brilliant Green Medium as the indicator medium.

50 Novobiocin Discs (Code FT207).
Each disc contains 1.8mg of novobiocin.

2 Syringes and needles

1 Spanner (Code FT202)

50 Labels

1 Instruction leaflet

Materials Required but not Supplied:
Pre-enrichment medium

Sterile distilled water

Salmonella Rapid Test Elective Medium (SRTEM) (Code CM857)

Pipettes

Salmonella Latex Test (Code FT203)

Vortex mixer

Incubators (35°C or 37°C and 41°C)

Preparation of the test material
Test samples are pre-enriched in a suitable medium, for example by homogenisation of a 1 in 10 dilution of the sample in Buffered Peptone Water (Code CM509) or other recommended media. Incubate the pre-enrichment culture at 35°C for 18 hours.

For full procedure please see product insert.

References

1 *Compendium of Methods for the Microbiological Examination of Foods*, 2nd Ed. (1984), American Public Health Association Inc, Ed. M.L. Speck.
2 *F.D.A. Bacteriological Analytical Manual*, 6th Ed. (1984). Published by Association Official Analytical Chemists.
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4 Personal Communication from Dr. A.C. Baird-Parker.
5 *Rapid Detection of Salmonellae in Foods – A Convenient two d Procedure*. Letters in Applied Microbiol., 1989, 8, 139–142. R. Holbrook, J.M. Anderson, A.C. Baird-Parker, L.M. Dodds, D. Sawhney, S.H. Stutchbury and D. Swaine.
6 *Comparative Evaluation of the Oxoid Salmonella Rapid Test with three other Rapid Salmonella Methods*. Letters in Applied Microbiol., 1989, 9, 161–164. R. Holbrook, J.M. Anderson, A. Baird-Parker and S.H. Stuchbury.

S.P.R.I.N.T.

Oxoid S.P.R.I.N.T. (Simple Pre-enrichment and Rapid Isolation New Technology) Salmonella Kit is a rapid 24-hour enrichment method for the isolation of Salmonella from foods.

The kit allows traditional pre-enrichment in Buffered Peptone Water (BPW) and selective enrichment in Rappaport-Vassiliadis (RV) to be performed in one 24-hour step. The kit contains the following:

S.P.R.I.N.T. Salmonella Recovery Supplement
The supplement allows all damaged Salmonella to recover within 6 hours reducing the time required in S.P.R.I.N.T. Salmonella Enrichment Broth.

S.P.R.I.N.T. Salmonella Time Release Capsules
These burst open after approximately 6 hours releasing a mixture of selective agents into the Enrichment Broth.

Product	Pack Size	Order Code
S.P.R.I.N.T. Salmonella Kit	20 tests	FT601A
S.P.R.I.N.T. Salmonella Enrichment Broth	500g	CM966B

an enrichment broth specifically formulated for use with S.P.R.I.N.T. Salmonella Kit.

Novobiocin Supplement 10 vials SR181E for use with heavily contaminated samples.

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TOXIN DETECTION KITS

November 1998

TOXIN DETECTION KITS

These products detect a wide variety of bacterial toxins and enterotoxins in food, faecal or culture samples.

The kits are simple to operate but are reliable for toxin detection in the majority of laboratories where sophisticated equipment or special skills in chemistry are not available.

Both industrial and clinical applications benefit from their use. Improved quality control of foods and raw materials, better detection of food poisoning outbreaks and rapid clinical diagnosis are examples of these benefits. The ability to carry out such tests in the laboratory, without sending them to external reference laboratories, is an additional benefit.

RPLA BACTERIAL TOXIN DETECTION

Bacterial enterotoxins can be detected by performing a simple dilution assay, using reverse passive latex agglutination (RPLA). In a *standard* agglutination assay, soluble antibody reacts with particulate antigen such as bacterial cells. However, in a REVERSED agglutination assay the antibody, which is attached to particles, reacts with the soluble antigen. The particles (in this case, latex) do not themselves play a part in the reaction and they are therefore PASSIVE. The cross-linking of the latex particles by the specific antigen/antibody reaction results in the visible LATEX AGGLUTINATION reaction. The test is performed in a microtitre plate, using antitoxin-coated latex particles which agglutinate in the presence of toxin to give clear visual readings, after 20 to 24 hours incubation at room temperature.

Enterotoxins of *Staphylococcus aureus, Vibrio cholerae, Escherichia coli, Clostridium perfringens* and *Bacillus cereus* can be detected with these tests by any laboratory.

Escherichia coli produces two types of enterotoxin: a heat-labile (LT) and a heat-stable enterotoxin (ST). The former may be isolated and identified using the RPLA test described above; the latter, however, by means of the *E. coli* ST EIA kit, a competitive enzyme immunoassay. Both kits provide a rapid and effective means of detection.

SET-RPLA

Code: TD900

A kit for the detection of staphylococcal enterotoxins A, B, C and D in food samples or culture filtrates by RPLA.

Staphylococcal enterotoxin food poisoning is caused by the growth of *Staph. aureus* in the food but it cannot be accurately diagnosed by bacterial cultivation. Some strains of *Staph. aureus* are not toxin-producers and some subsequent food treatment may destroy the staphylococci without affecting the pre-formed toxin. By direct testing for the toxin in the suspected food, the specific cause of food poisoning can be identified. Toxin-producing strains of *Staph. aureus* isolated in the food preparation environment can also be identified by their specific toxin type and linked with the food sample.

VET-RPLA

Code: TD920

A kit for the detection of Vibrio cholerae enterotoxin and Escherichia coli heat-labile (LT) enterotoxin in culture filtrates by RPLA.

Enterotoxigenic *E. coli* (ETEC) strains are a common cause of diarrhoea in developing countries and of travellers moving between countries. The LT toxin can easily be detected in culture filtrates of the suspected strains. *V. cholerae* enterotoxin (CT) is antigenically similar to LT toxin and can be detected more easily because of the higher level of toxin produced in culture filtrates.

[See *E. coli* ST EIA (Code TD700) for the detection of ST enterotoxin]

PET-RPLA

Code: TD930

A kit for the detection of Clostridium perfringens enterotoxin in faecal samples or culture filtrates by RPLA.

The detection of *Cl. perfringens* toxin in faecal samples will, in the absence of other definitive evidence, show the specific cause of food poisoning from foods in which the organism has grown. The test also detects the minority of toxin-producing strains of *Cl. perfringens* which may be isolated from the food environment and the victim.

TST-RPLA

Code: TD940

A kit for the detection of staphylococcal toxic shock toxin in culture filtrates by RPLA.

The syndrome of staphylococcal toxic shock has been associated with certain toxin-producing strains of *Staph. aureus*.

Detection of these strains of staphylococci can be reliably carried out using this test.

BCET-RPLA

Code: TD950

A kit for the detection of Bacillus cereus enterotoxin (diarrhoeal type) in foods and culture filtrates by RPLA.

Diarrhoeal food poisoning caused by *B. cereus* enterotoxin can be diagnosed directly by detection of the specific toxin in the suspected food or indirectly from culture filtrates of *B. cereus* isolated from the food environment or patient.

The acute onset ''emetic-syndrome'' toxin associated with cooked rice will not be detected with this kit.

E. COLI ST EIA

Code: TD700

A kit for the detection of E. coli heat-stable (ST) enterotoxin in culture filtrates or supernatants by enzyme immunoassay (EIA).

Enterotoxigenic *E. coli* (ETEC) are a common cause of infantile diarrhoea in developing countries and a cause of traveller's diarrhoea. These ETEC strains may produce heat-labile enterotoxin (LT) or heat-stable enterotoxin (ST) or both of these. This kit together with VET-RPLA (Code TD920) will determine the toxin production of any suspected ETEC strain.

The EIA test may be carried out on culture filtrates or supernatants. Performed in microtitre wells, the test is a competitive EIA technique with solid-phase toxin coated on the wells. The toxin used is a pure synthetic *E. coli* heat-stable preparation. The antibody-enzyme conjugate is composed of a monoclonal antibody linked to horseradish peroxidase. This conjugate will bind to the solid-phase toxin or to the toxin in the culture fluid. The presence of toxin in the culture fluid will compete with the solid-phase toxin and thus reduce the solid-phase binding of conjugate. After washing the well, such competition will result in a reduced colour intensity on addition of the substrate, which is clearly distinguished from a colourful negative result.

VTEC-RPLA

Code: D960

A reverse passive latex agglutination test for the detection of verocytotoxins VT1 and VT2 produced by Escherichia coli cultured from food and faecal samples.

INTRODUCTION

Verocytotoxin producing *E. coli* (VTEC) are transmitted through food, water and person-to-person contact, and are known to cause a range of illnesses from self-limiting watery diarrhoea and haemorrhagic colitis to haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura. These illnesses can be fatal, making the increasing incidence of VTEC contamination a cause for widespread concern.

Unlike other tests which detect the presence of strains such as *E. coli* O157 (of which some, but not all, produce verocytotoxins), the Oxoid VTEC-RPLA test detects the toxins themselves, providing a clear and specific indication of VT1 or VT2 production. This overcomes the problem of positive results from other latex and culture assays (i.e. those which detect the organism rather than the toxin) where non-toxin-producing *E. coli* 0157 strains are present. Similarly, it also overcomes the problem of negative results in cases where non-0157 strains are responsible for toxin production.

The test can be used with isolates cultured from both food and faecal samples.

PRINCIPLES OF THE ASSAY

Polymer latex particles are sensitised with purified rabbit anti-serum which is reactive either with *E. coli* verocytotoxin VT1 or VT2. The latex particles will agglutinate in the presence of one or both of the *E. coli* verocytotoxins. Agglutination results in the formation of a lattice structure. On setting, this forms a diffuse layer on the base of a V-bottom microtitre well. If *E. coli* verocytotoxin is absent, or at a concentration below the assay detection level, no such lattice structure can be formed, and a tight button will therefore be observed. The use of polymyxin B solution facilitates the release of verocytotoxins.

C. DIFFICILE TOXIN A TEST

TD970

C. difficile Toxin A Test is a rapid immunoassay for the direct qualitative detection of Clostridium difficile toxin A in faecal samples.

INTRODUCTION

Clostridium difficile is a Gram-positive anaerobic bacillus which has been identified as a common nosocomial pathogen that causes diarrhoea and pseudomembranous colitis associated with antibiotic therapy. In the 1960's and 70's, antibiotic associated pseudomembranous colitis became a major clinical problem, particularly due to the use of broad spectrum antibiotics such as ampicillin and cephalosporins. Larson *et al* (1977) reported that stool samples from affected patients contained a toxin that produced cytopathic changes in tissue culture cells[1]. Subsequently, *C. difficile* was identified as the organism responsible for toxin production[2,3].

C. difficile is known to produce at least two toxins, designated A and B. Toxin A (mwt. 308 Kda) causes fluid secretion, mucosal damage and internal inflammation. Toxin B (mwt. 250 Kda) is approximately 1000 fold more potent as a cytotoxin than toxin A, but is not enterotoxic[4]. It is therefore accepted that toxin A plays a more important role in the pathogenesis of *C. difficile*[5].

Both toxins A and B are produced at the same time. The symptoms are primarily due to toxin A, but toxin B is thought to play a role in the disease by acting synergistically with toxin A[6].

When established in the colon, *C. difficile* produces the toxins which cause diarrhoea and pseudomembranous colitis. *C. difficile* infection is almost exclusively a nosocomial infection. Outbreaks once established, are difficult to control and re-infection of patients is common.

Laboratory diagnosis depends on demonstrating the presence of *C. difficile* toxins in the stool sample. The most widely used test for detection of *C. difficile* toxin is cell cytotoxicity with neutralisation (using, for example, vero cells) which primarily detects toxin B[7]. This test takes up to 48 hours to obtain a result.

The Test Unit provides a rapid 30 minute method for the detection of *C. difficile* toxin A from patient samples, which is suitable for single or batch testing.

The Test Unit utilises a unique technology. The test is performed by addition of the sample to the absorbent pad in the Sample Window.

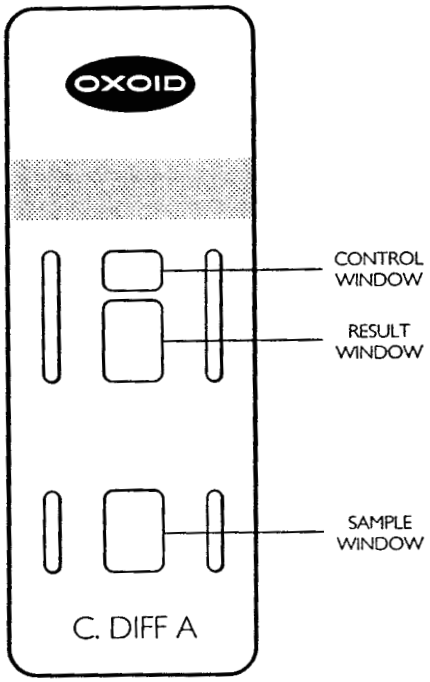
The absorbent pad incorporates a latex-labelled monoclonal antibody to *Clostridium difficile* toxin A. The pad is in contact with a test strip which contains regions of immobilised antibody.

To perform the test, the user delivers 125µl of sample to the Sample Window, thus saturating the absorbent pad. The sample moves from the pad to the test strip, mobilising anti *C. difficile* toxin A mouse monoclonal antibody attached to blue latex beads present in the pad.

The sample, antibody-latex and *C. difficile* toxin A antibody-latex continues to move up the test strip to an immobilised region of anti *C. difficile* toxin A antibody.

The *C. difficile* toxin A attaches to the immobilised antibody and a blue line become visible in the Result Window.

As the sample continues to move up the test strip, it comes into contact with an immobilised strip of rabbit anti-mouse antibody, forming a blue line in the Control Window.



TEST PRINCIPLE

Clostridium difficile toxin A is extracted from faecal samples as described in ''Sample Preparation''.

The Test Unit contains monoclonal antibodies to *C. difficile* toxin A.

The extracted antigen is added to the pad in the Sample Window. The pad contains blue latex labelled with antibody. The extract rehydrates the complex and, if present, the specific antigen reacts with the antibody.

The antibody-antigen-latex complex travels through the test strip by capillary action to an area midway along the Result Window. This area contains an immobilised line of monoclonal antibody to *C. difficile* toxin A.

A further reaction between the antigen-antibody-latex complex and the fixed antibody is shown by the formation of a blue line in the Result Window.

If no *C. difficile* toxin A is present no line will appear in the Result Window.

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