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Microscopy

Löffler's methylene blue solution

for microscopy

IVD In Vitro Diagnostic Medical Device



This "Löffler's methylene blue solution - for microscopy" is used for human-medical cell diagnosis and serves the purpose of the bacteriological and histological investigation of sample material of human origin. It is a ready-to-use staining solution that when used together with other in vitro diagnostic products from our portfolio makes target structures (e.g. acid-fast mycobacteria, by fixing, embedding, staining, counterstaining, mounting) in bacteriological and histological specimen materials, for example smears of enriched bacterial cultures or histological sections of e.g. the lung, evaluable for diagnostic purposes.

Principle

The cell wall of mycobacteria has a high proportion of wax and lipids and hence absorbs dyes only very slowly. The most efficient staining method is the hot staining according to Ziehl-Neelsen. In this method, carbolfuchsin solution is applied to the specimen and heated. This heating process accelerates the rate at which the fuchsin dye is absorbed and thus also that of the formation of the mycolate-fuchsin complex in the cell wall.

Once the mycobacteria have absorbed the fuchsin dye, it is virtually impossible to decolorize them again, even when they are intensively treated with a decolorizing solution such as e.g. hydrochloric acid in ethanol. Accordingly, mycobacteria are termed as acid- and alcohol-fast for staining, and are stained red in the microscopic visualization. Correspondingly, all non-acid-fast microorganisms are counterstained with an appropriate dye. In the present operating instructions is counterstained accordance with methylene blue.

Pretreatment of the specimens with Sputofluor® dissolves the bacteria from the surrounding viscid sputum and cell material. Sputofluor® also has a disinfectant effect, with the result that any microorganisms that are present are killed off.

Sample material

Smears of bacteriological material that have been air-dried, heat-fixed, and pretreated with Sputofluor® like sputum, smears from fine needle aspiration biopsies (FNAB), rinses, imprints, effusions, pus, exsudates, liquid and solid cultures Sections of formalin fixed, paraffin embedded tissue (3 - 4 µm thick paraffin sections)

Reagents

Cat. No. 101287
Löffler's methylene blue solution for microscopy 100 ml, 500 ml, 2.5 l

Also required:

Cat. No. 109215 Ziehl-Neelsen carbolfuchsin solution for microscopy 100 ml, 500 ml, 2.5 l
Cat. No. 100327 Hydrochloric acid in ethanol for microscopy 1 l, 5 l

Alternatively:

Instead of the combination of single reagents, the staining kit 1.00497.0001 can be used:

Cat. No. 1.00497.0001
Tb-color modified 1 set
Staining kit for the detection of mycobacteria (AFB) by hot staining method

Sample preparation

The sampling must be performed by qualified personnel.

Sputum

The mycobacteria should be pretreated with Sputofluor® to dissolve them from mucus and cellular structures. In this process, the active ingredient hypochlorite dissolves the organic material by oxidation and gently releases the mycobacteria so that they can be processed further.

Reagent preparation: Preparation of Sputofluor® solution 15 %

For preparation of approx. 100 ml solution mix:

Sputofluor®	15 ml
Distilled water	85 ml

Preparing sample material in centrifuge tubes:	
Sample	1 part (min. 2 ml)
Sputofluor® solution (15 % in distilled water)	3 parts
Shake vigorously	10 min
Centrifuge at 3000 - 4800 rpm	20 min
Decant supernatant Prepare smears of the sediment Air-dry	

Punctates, lavages, sediments

After appropriate enrichment measures, smear sample material on the slide and allow to air-dry.

Histological sections

Löffler's methylene blue solution can be used to stain histological sections. Deparaffinize sections in the conventional manner and rehydrate in a descending alcohol series. Pretreatment with Sputofluor® is not necessary for specimens fixed with formalin.

Fixation

Specimens are fixed over a Bunsen burner flame (2 - 3 times, taking care to avoid excessive heating). The specimens can also be fixed by heating at 100 - 110 °C in a drying cabinet or on a heating plate for 20 min. Excessive temperatures or prolonged heating may involve a deterioration of the staining performance.

All samples must be treated using state-of-the-art technology.

All samples must be clearly labeled.

Suitable instruments must be used for taking samples and their preparation. Follow the manufacturer's instructions for application / use.

Reagent preparation

The Löffler's methylene blue solution - for microscopy used for staining is ready-to-use, dilution of the solution is not necessary and merely produces a deterioration of the staining result and their stability.

Procedure

Staining on the staining rack

The stated times should be adhered to to guarantee an optimal staining result.

Slide with fixed smear		
Ziehl-Neelsen carbolfuchsin solution	cover completely, carefully heat three times from below with the Bunsen burner until steam forms Do not allow the staining solution to boil!	stain for 5 min in total
Tap water	rinse until no further clouds of dye are produced	
Hydrochloric acid in ethanol	cover completely and leave to react	15 - 30 sec*
Tap water	rinse immediately	
Löffler's methylene blue solution	counterstaining, cover completely and leave to react	30 sec**
Tap water	rinse carefully	
Air-dry (e.g. over night or at 50°C in the drying cabinet)***		

* depending on thickness of specimen

** or 1 min with diluted Löffler's methylene blue solution (dilution: 1:10 (1+9) with dist. water)

*** Histological samples are not air-dried, after dehydration (ascending alcohol series) and clarification with xylene or Neo-Clear®, they can be mounted with water-free mounting agents (e.g. Neo-Mount®, Entellan®, DPX Neu, or Entellan® Neu) and a cover glass and can then be stored.

Covering with non-aqueous mounting media (e.g. Neo-Mount®, Entellan®, DPX new, or Entellan® new) and a cover glass is recommended for the storage of bacteriological specimens for several months. For this purpose, the stained specimens must be dried very well. When left unmounted, the stain remains stable for approx. 3 days, covered with immersion oil for just a few hours.

The use of immersion oil is recommended for the analysis of stained slides with a microscopic magnification >40x.

Result

Mycobacteria red
Background blue

Evaluation

A positive result means "acid-fast rod bacteria present", a negative result "acid-fast rod bacteria not present". It is not possible to tell whether the bacteria found are *Mycobacterium tuberculosis* or of a different species of mycobacterium. The vitality (active, inactive) of the bacteria can also not be determined.

In the event that mycobacteria are detected, further examinations should be performed in specialized laboratories.

Trouble-shooting

Fixing

A sufficient degree of heat-fixing using a Bunsen burner or in a heating cabinet is essential to prevent the infectious potential of the specimens and further proliferation of the bacteria.

No staining of mycobacteria

The critical step of the mycobacteria-staining process is the decolorizing step, which can be influenced by the thickness of the specimen smear. In addition, a freshly prepared solution of hydrochloric acid in ethanol is highly reactive, meaning that the result should be evaluated with caution. The incubation times stated in this protocol should be kept accurately in the decolorizing step, since otherwise false-negative results may ensue.

Technical notes

The microscope used should meet the requirements of a medical diagnostic laboratory.

When using automatic staining systems, please follow the instructions for use supplied by the supplier of the system and software.

Remove surplus immersion oil before filing.

Diagnostics

Diagnoses are to be made only by authorized and trained personnel.

Valid nomenclatures must be used.

Further tests must be selected and implemented according to recognized methods. Suitable controls should be conducted with each application in order to avoid an incorrect result.

Storage

Store the Löffler's methylene blue solution - for microscopy at +15 °C to +25 °C.

Shelf-life

The Löffler's methylene blue solution - for microscopy can be used until the stated expiry date.

After first opening of the bottle, the contents can be used up to the stated expiry date when stored at +15 °C to +25 °C.

The bottles must be kept tightly closed at all times.

Capacity

approx. 250 stainings / 500 ml

Additional instructions

For professional use only.

In order to avoid errors, the application must be carried out by qualified personnel only.

National guidelines for work safety and quality assurance must be followed.

Microscopes equipped according to the standard must be used.

If necessary use a standard centrifuge suitable for medical diagnostic laboratory.

Protection against infection

Effective measures must be taken to protect against infection in line with laboratory guidelines.

Instructions for disposal

The package must be disposed of in accordance with the current disposal guidelines.

Used solutions and solutions that are past their shelf-life must be disposed of as special waste in accordance with local guidelines. Information on disposal can be obtained under the Quick Link "Hints for Disposal of Microscopy Products" at www.microscopy-products.com. Within the EU the currently applicable REGULATION (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 applies.

Auxiliary reagents

Cat. No.	100327	Hydrochloric acid in ethanol for microscopy	1 l, 5 l
Cat. No.	100497	Tb-color modified Staining kit for the detection of mycobacteria (AFB) by hot staining method	1 set
Cat. No.	100579	DPX new non-aqueous mounting medium for microscopy	500 ml
Cat. No.	104699	Immersion oil for microscopy	100-ml dropping bottle, 100 ml, 500 ml
Cat. No.	107960	Entellan® rapid mounting medium for microscopy	500 ml

Cat. No.	107961	Entellan® new rapid mounting medium for microscopy	100 ml, 500 ml, 1 l
Cat. No.	108000	Sputofluol® for microbiology and microscopy	1 l
Cat. No.	108298	Xylene (isomeric mixture) for histology	4 l
Cat. No.	109016	Neo-Mount® anhydrous mounting medium for microscopy	100-ml dropping bottle, 500 ml
Cat. No.	109215	Ziehl-Neelsen carbolfuchsin solution for microscopy	100 ml, 500 ml, 2,5 l
Cat. No.	109843	Neo-Clear® (xylene substitute) for microscopy	5 l

Safety classification

Cat. No. 101287

Please observe the hazard classification printed on the label and the information given in the safety data sheet.

The safety data sheet is available on the website and on request.

Main components of the product

Cat. No. 101287

C.I. 52015	4.2 g/l
C ₂ H ₅ OH	190 g/l
pH	8.0 - 8.6
1 l =	0.97 kg

Other IVD products

Cat. No.	105174	Hematoxylin solution modified acc. to Gill III for microscopy	500 ml, 1 l, 2.5 l
Cat. No.	109093	TB-fluor Staining kit for fluorescence-microscopic detection of acid fast bacteria	6x 500 ml
Cat. No.	109204	Giemsa's azur eosin methylene blue solution for microscopy	100 ml, 500 ml, 1 l, 2.5 l
Cat. No.	109844	Eosin Y-solution 0.5% aqueous for microscopy	1 l, 2.5 l
Cat. No.	111609	Histosec® pastilles soldification point 56-58°C embedding agent for histology	1 kg, 10 kg (4x 2.5 kg), 25 kg
Cat. No.	111885	Gram-color stain set for the Gram staining method	1 set
Cat. No.	115161	Histosec® pastilles (without DMSO) soldification point 56-58°C embedding agent for histology	10 kg (4x 2.5 kg), 25 kg
Cat. No.	116450	Tb-color staining kit for the microscopic investigation of mycobacteria (cold staining)	1 set

Literature

1. Romeis - Mikroskopische Technik, Editors: Mulisch, Maria, Welsch, Ulrich, 2015, Springer-Verlag Berlin Heidelberg
2. Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble, 6th Edition
3. Theory and application of Microbiological Assay, Hewitt, W. and Vincent, S., 1989, Academic Press
4. Conn's Biological Stains: A Handbook of Dyes, Stains and Fluorochromes for Use in Biology and Medicine, 10th Edition, (ed. Horobin, R.W. and Kiernan, J.A). Bios, 2002



Consult instructions for use



Manufacturer



Catalog number



Batch code



Caution, consult accompanying documents



Use by YYYY-MM-DD



Temperature limitation

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