MUELLER HINTON II AGAR + HORSE BLOOD + 20mg/L NAD (MH-F).

INSTRUCTIONS FOR USE THE READY-TO-USE PLATED MEDIUM

1. Intended use

Mueller Hinton II Agar + Horse Blood +20mg/L NAD is a medium designed to test drug susceptibility according to EUCAST procedures by the diffusion-agar method, as well as resistance mechanisms of fastidious aerobic bacteria (streptococci) isolated from human clinical specimens.

The function of the medium is to support diagnosis by determining the antimicrobial susceptibility/resistance profile of fastidious bacteria isolated from human clinical specimens.

Information on the drug susceptibility profile and determination of the resistance mechanism of the pathogen detected in the patient's clinical specimen allows to make an appropriate, effective antibiotic therapy, suited to an individual patient.
The EUCAST disk-diffusion method is based on a method described by the International Collaborative Study of Antimicrobial Susceptibility Testing in 1972. Due to its simplicity of implementation, it is the most widely used method of testing bacterial drug susceptibility in medical laboratories. Correct, standardized performance of the test according to the EUCAST method and obtaining reliable results requires the use of this method without modification, including the use of the medium specified by EUCAST.
According to EUCAST guidelines, Mueller Hinton II Agar + Horse Blood + 20mg/L NAD is a medium designed for determining the drug susceptibility profile of fastidious bacteria, especially *Streptococcus* spp. (including *S. pneumoniae*), *Haemophilus influenzae, Moraxella catarrhalis, Listeria monocytogenes, Campylobacter jejuni and coli, Pasteurella multocida, Corynebacterium spp, Aerococcus sanguinicola and urinae and Kingella kingae.*

Cat. no:	**Medium type:**	**Packaging:**
1370PD90; 201370	Solid medium on a plate	1x10 pcs (90 mm)

2. Principle of the procedure

Beef extract and acid casein hydrolysate are sources of nitrogen, vitamins, carbon and amino acids. Corn starch absorbs toxic metabolic products. Defibrinated horse blood and NAD enable the growth of fastidious bacteria. Agar is a solidifying agent. Drug susceptibility testing of microorganisms should be performed by the disk-diffusion method in accordance with the current EUCAST guidelines.
The disk-diffusion method that uses the phenomenon of a concentration gradient of an antimicrobial drug forming in a solid medium, as a result of its diffusion from a blotting paper disk, which inhibits the growth of microorganisms around the disk. The diameter of the zone of inhibited growth, measured in millimeters, is compared with the corresponding limit value specified in the relevant EUCAST guidelines. The result of this comparison allows the tested microorganism to be classified into the appropriate susceptibility category to the specific antimicrobial drugs used in the test. In order to determine the mechanism of resistance, disks containing specific antimicrobial drugs are arranged on the medium. The characteristic size and shape of the zones inhibition of bacterial growth on the medium allows to determine the presence and type of resistance mechanism of the tested pathogen.

3. Medium composition

In g/l distilled water:		**Supplements/Liter:**	
Casein peptone	17,5 g	Horse blood	50 ml
Corn starch	1,5 g	NAD	0.02 g
Beef extract	2,0 g		

Agar	17,0 g	

pH 7.3± 0.1 at 25° C.
Appearance of the medium – Homogeneous, red.

4. Medium preparation

The medium is ready to use. Bring the medium to room temperature immediately before use.

5. Equipment required, not provided

Equipment and reagents necessary for the test (e.g., saline, sterile swabs, antibiotic-soaked blotting paper disks) and standard microbiological laboratory equipment, including a bacteriological densitometer or density standard, an incubator and a ruler or another devices for measuring the zones of inhibited growth.

6. Precautions

- The product is intended for professional use only.
- Non-automated product.
- The medium contains components of animal origin, which may be associated with the presence of biological pathogens, therefore must be handled in accordance with principles of handling potentially infectious biological material.
- Do not use plates if the medium shows signs of microbial contamination, discoloration, drying, cracking or other signs of deterioration.
- Do not use damaged plates.
- Do not use plates after the expiration date.
- Re-incubation of previously inoculated plates is not allowed.
- To ensure correct test results, follow these instructions and the EUCAST procedure.
- If the handling of the medium differs from that described in this manual, the laboratory is obliged to validate the procedure adopted.

7. Storage

Store plates at 2-12ºC until the expiration date. Store plates in their original packaging in an inverted position, away from direct light sources. To avoid freezing of agar, do not store plates close to the refrigerator walls. To avoid appearance of water condensation on the plate lid, do not open the refrigerator more often than necessary and do not store plates in an overfilled refrigerator.

8. Expiration date

The medium stored at 2-12ºC retains its properties for up to 45 days from the manufacture date.

9. Specimen type

The material for the tests are pure (about 16-24 hours) culture of pathogenic strain of fastidious bacteria isolated from human clinical specimens, or other samples inoculated onto solid medium.

10. Test procedure

Current EUCAST procedures and guidelines must be strictly followed, to ensure correct, reliable disk-diffusion susceptibility testing results

1. Allow the medium to warm to room temperature before inoculation.

2. Inoculum preparation

Prepare a 0.5 McFarland suspension of the test strain by suspending colonies of the strain in saline solution.
Collect colonies with a sterile loop or swab from non-selective medium, after 18-24 hours of incubation.

Select a few morphologically similar colonies. Determine the density of the inoculum using a bacteriological densitometer. The density of the suspension can also be determined by macroscopically comparing the density of the test strain's suspension with a 0.5 McFarland density standard. In this case, the turbidity of the test strain's suspension to the density standard should be compared on a white background with black stripes.

The prepared suspension of the test strain should be used within 15 minutes, and no later than 60 minutes after preparation.

3. **Preparation of bacterial lawn**

Dip a sterile cotton swab into the prepared suspension of the test strain. For Gram-negative bacteria, to avoid excessive inoculation, remove the excess suspension from the swab by pressing it against the inside of the tube. For Gram-positive bacteria, there is no need to press the swab against the inside of the tube. Media can be inoculated manually or with an automatic inoculator. Spread the suspension evenly over the entire agar surface, making sure there are no gaps between each band, which is especially important for Gram-positive bacteria.

4. **Apply antibiotic disks**

Apply antibiotic paper disks to the agar surface. The disks should be applied to the medium within 15 minutes of inoculation. Press the disks lightly, as they should completely adhere to the agar surface. Once applied, the disks must not be moved, due to the rapid diffusion of the antibiotic from the disk into the medium. The number of disks on the plate should be limited, so that the resulting zones of inhibition do not overlap and individual antibiotics do not interact with each other. A maximum of 6 antibiotic disks can be applied to a 90 mm diameter plate.

5. Incubation

Incubate plates under aerobic conditions at the temperature and for the time specified in EUCAST manual, depending on the type of tested microorganism. The plates should be in an inverted position (agar side up), while making sure that the antibiotic disks have not fallen off the agar surface. Incubation of the plates should begin within 15 minutes of applying the disks. The plates should not be incubated for a period longer than recommended.

Detailed guidelines for the selection of antibacterial drugs and the performance of drug susceptibility testing by the disk-diffusion method are available in current EUCAST manuals.

11. Reading and interpretation

After incubation, measure the size of the inhibition zones using a calibrated instrument such as a ruler or caliper, or use an automatic system to measure the size of the inhibiting zone.
Interpret the obtained results based on current EUCAST guidelines.

After incubation:

- Examine plates for the bacterial lawn.
- Measure the diameter or inhibition zones according to current EUCAST guidelines using a ruler or other measuring instrument in millimeters.
- Interpret the obtained measurements based on current EUCAST guidelines, assigning the appropriate drug susceptibility category for each antimicrobial drug used.

When testing a pathogen for resistance mechanisms, assess the size and characteristic shape of inhibited bacterial growth should also be assessed.

12. Quality control

Perform medium quality control at a frequency and in a manner consistent with current EUCAST procedures for quality control of the disk-diffusion method and laboratory procedures.
Reference strains that ensure measurement consistency in accordance with EUCAST procedures should be used to perform quality control tests.

13. Limitations of the method

- Numerous factors can affect the propriety of the size of zones of inhibited growth and the results of the tests.

- Numerous factors can affect the size of inhibition zones and the results of drug susceptibility testing, such as bacterial suspension density, growth rate, medium composition and pH.
- Drug susceptibility testing by the disk-diffusion method should be performed only with pure bacterial cultures, around 16-24 hours old.
- If the inoculum density is too high, it can reduce the diameter of inhibition zones, and if it is too low, it can increase the size of growth inhibition zones and cause difficulties in measuring them.
- Leaving inoculated plates at room temperature for longer than the recommended period before applying the antibiotic disks may cause microbial proliferation, resulting in a decrease in the diameters of the zones of inhibition. Therefore, it is important to follow the 15-15-15 rule: the suspension should be used within 15 minutes of preparation, the disks should be applied within 15 minutes of inoculation, and plate incubation should begin within 15 minutes of disk application.
- Improper storage of antibiotic disks can affect the stability of the tested antibiotics in them, which can reduce the diameter of the zones of inhibition and can be a source of interpretive errors in assessing the drug susceptibility of the pathogen under study.
- An important factor affecting the test result is the arrangement of stacks of inoculated plates in the incubator and if it allows the heat to speed evenly. A maximum recommended number of plates in a stack is 5.
- Excessive shrinkage of the medium, due to improper storage can lead to false results.
- Improper arrangement of antibiotic disks to test on the medium may result in false results

14. Characteristics of the method

Presented in EUCAST documents and available literature.

15. Disposal of used material

Used and unused materials should be disposed of in accordance with current medical waste handling regulations and laboratory procedures for the disposal of infectious and potentially infectious materials.

16. Reporting of adverse events

According to current regulations, adverse events and incidents that can be directly linked to the medium described in this manual must be reported to the manufacturer and to the competent authorities.

17. References

History of document changes

Date of change	Section	Description of the change
2023/02/15	Entire document	Adaptation to the requirements of EU Regulation 2017/746

NOTE
The revision history of the document does not include editorial changes.

SYMBOL	NAME OF SYMBOL	DESCRIPTION	REF.
	Manufacturer	Indicates the medical device manufacturer.	5.1.1
	Date of manufacture	Indicates the date after which the medical device is not to be useed.	5.1.3

	Catalogue number	Indicates the manufacturer's catalogue number so that the medical device can be used..	5.1.6
	Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified.	5.1.5
	In vitro diagnostic medical device	Indicates a medical device that is intended to be used as an invitro diagnostic medical device.	5.5.1
	Do not re-use	Indicates a medical device that is intended for one single use only.	5.4.2
	Contains sufficient for <n> tests	Indicates the total number of tests that can be performed with the medical device.	5.5.5
	Use –by date	Indicates the date after which the medical device is not to be used	5.1.4
	Temperature limit	Indicates the temperature limits of temperature shall be indicates adjacent to the upper and lower horizontal lines.	5.3.7
	Safety symbol (Compliance with EU requirements)	The CE marking on a product is a manufacturer's declaration that the product complies with the essential requirements of the relevant European Union health, safety and environmental regulations.	nd.
	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use.	5.4.3
	Sterilized using aseptic processing techniques	Indicates a medical device that has been manufactured using accepted aseptic techniques.	5.2.2

	Do not use if package is damaged and consult instructions for use	Indicates that a medical device that should not be used if the package has been damaged or opened and that the user should consult the instructions for use for addictional information.	5.2.8
	Contains biological material of animal origin	Indicates a medical device that contains biological tissue, cells, or their derivatives, of animal origin	5.4.8