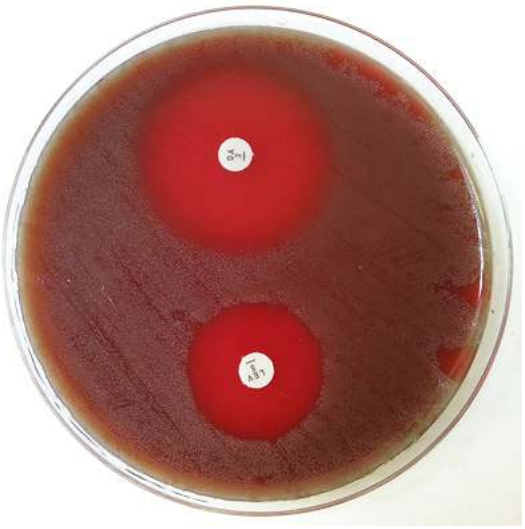
INSTRUCTIONS FOR USE

MUELLER HINTON AGAR F (MHA-F)

Ready-to-use plates



Mueller Hinton Agar F: *S.pneumoniae* ATCC 49619

1 - INTENDED USE

In vitro diagnostic device.
Culture medium for Antimicrobial Susceptibility Testing (AST) by disk diffusion method of fastidious organisms.

2 - COMPOSITION - TYPICAL FORMULA *

Beef extract	2.0 g
Acid digest of casein	17.5 g
Starch	1.5 g
Agar	17.0 g
Defibrinated horse blood	50 ml
β-NAD	20 mg
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The development of bacterial resistance to antimicrobials in the first half of the twentieth century, resulted in the need for physicians to request the microbiology lab to test a patient's pathogen against various concentrations of a given antimicrobial to determine susceptibility or resistance to that drug.[1] The culture medium proposed for the Kirby-Bauer method was Mueller Hinton Agar, originally developed by Howard Mueller and Jane Hilton in 1941 for the isolation of gonococcus and meningoccus.[2]
Currently, the Clinical Laboratory Standards Institute (CLSI) for USA and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) for Europe are responsible for updating and modifying the original procedure through a global consensus process.[3,4] Interpretative guidelines for inhibition zone sizes are included in their publications.[3,5]
Mueller Hinton Agar supplemented with defibrinated horse blood and β-NAD (MHA-F) is recommended and standardized by EUCAST[4] for testing fastidious organisms such as *Streptococcus pneumoniae*, Viridans group streptococci, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Listeria monocytogenes*, *Pasteurella multocida Campylobacter jejuni* and *coli*, *Corynebacterium* spp., *Aerococcus sanguinicola* and *urinae*, *Kingella kingae, Aeromonas* spp., *Burkholderia pseudomallei, Campylobacter jejuni* and *coli*.
Defibrinated horse blood and β-NAD enable the growth of fastidious bacteria with minimal interference in the results of the antimicrobial susceptibility test.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	brilliant red
Final pH at 20-25°C	7.3 ± 0.1
Agar depth	4.0 ± 0.5 mm

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Mueller Hinton Agar F (MHA-F)	Ready-to-use plates	541740F	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, controlled atmosphere generators and jars, antimicrobial susceptibility paper disks.

7 - SPECIMENS

AST by disk diffusion method is designed to be used with pure culture of strains isolated from clinical specimens.
Mueller Hinton Agar F is not intended for microbial isolation directly from clinical specimens.
A Gram stain and a preliminary bacterial identification are required for choosing the appropriate antimicrobial agents to be tested.
EUCAST has published a method for rapid AST (reading at 4, 6 or 8 h incubation) directly from positive blood culture bottles, validated for selected organisms; consult the EUCAST document for the test procedure, reading and interpretation of inhibition zones.[6]

8- TEST PROCEDURE

The test procedure and the reading and interpretation of inhibition zones here described are a summary of EUCAST documents.[4,5,7]

- The surface of the agar should be dry before use. No drops of water should be visible on the surface of the agar or inside the lid. If necessary, dry plates either at 20-25°C overnight, or at 35°C, with the lid removed, for 15 min. Do not over-dry plates.
- Use a sterile loop or a cotton swab to pick colonies from an overnight culture on non-selective media. Use several morphologically similar colonies (when possible) to avoid selecting an atypical variant. Suspend the colonies in saline and mix to an even turbidity. Adjust the density of the organism suspension to 0.5 McFarland by adding saline or more bacteria. The suspension must always be

used within 60 min of preparation. When *Streptococcus pneumoniae* is suspended from a chocolate agar plate, the inoculum must be equivalent to a 1.0 McFarland standard.
- Dip a sterile cotton swab into the suspension. To avoid over-inoculation of Gram-negative bacteria, remove excess fluid by pressing and turning the swab against the inside of the tube. For Gram-positive bacteria, do not press or turn the swab against the inside of the tube.
- Plates can be inoculated either by swabbing in three directions or by using an automatic plate rotator. Spread the inoculum evenly over the entire agar surface ensuring that there are no gaps between streaks.
- Allow disks to reach room temperature before opening cartridges or containers used for disk storage.
- Apply disks firmly to the surface of the inoculated agar plate within 15 minutes of inoculation. Disks must be in close and even contact with the agar surface and must not be moved once they have been applied as the initial diffusion of antimicrobial agents from disks is very rapid.
- The number of disks on a plate should be limited to avoid overlapping of zones and interference between agents. It is important that zone diameters can be reliably measured. The maximum number of disks depends on the organism and the selection of disks. Normally 6 and 12 disks are the maximum possible number on a 90 and 150 mm circular plate, respectively.
- Invert agar plates and make sure disks do not fall off the agar surface. Incubate plates within 15 min of disk application. If the plates are left at room temperature after disks have been applied, pre-diffusion may result in erroneously large zones of inhibition.
- Incubate at 35 ± 1°C in 4-6% CO_2 in air for 18 ± 2 h. Incubate *Aeromonas* spp. and *Burkholderia pseudomallei* at 35 ± 1°C in air for 18 ± 2 h. For *C.jeuni* and *coli*, incubate in microaerobic environment at 41±1°C for 24 hours.
- Isolates of *Corynebacterium, Aerococcus, Kingella* with insufficient growth after 16-20 h are re-incubated immediately and inhibition zones read after a total of 40-44 h incubation. Isolates of *Campylobacter* with insufficient growth are re-incubated for a total of 40-48 hours.

9 - READING AND INTERPRETATION

After incubation, read plates from the front with the lid removed and with reflected light.
A correct inoculum and satisfactorily streaked plates should result in a confluent lawn of growth. If individual colonies can be seen, the inoculum is too light and the test must be repeated.
The growth should be evenly distributed over the agar surface to achieve uniformly circular (non-jagged) inhibition zones.
Check that inhibition zones for quality control strains are within acceptable ranges.
For all agents, the zone edge should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye. Holding the plate at a 45-degree angle to the work bench may facilitate reading when zone edges are difficult to define.
Measure the inhibition zone diameters to the nearest millimetre with a ruler or a calliper.
For specific reading instructions consult the EUCAST document.[4] Interpret zone diameters into susceptibility categories according to the current breakpoint tables.[5]

10 - USER QUALITY CONTROL

All manufactured lots of Mueller Hinton Agar F plates are released for sale after the Quality Control has been performed to check the compliance with the specifications, according to EUCAST rules[4,6]. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Select the quality control strains specified by EUCAST and summarized here below, to monitor the performance of the test. Principal recommended control strains are typical susceptible strains, but resistant strains can also be used to confirm that the method will detect resistance mediated by known resistance mechanisms. Check that results for control strains are within acceptable ranges in EUCAST QC tables.[6] EUCAST recommended strains[4]:
- *Streptococcus pneumoniae* ATCC 49619 - Reduced susceptibility to benzylpenicillin
- *Haemophilus influenzae* ATCC 49766 - Susceptible, wild type
- *Campylobacter jejuni* ATCC 33560 – Susceptible, wild type
- *H.influenzae* ATCC 49247 – Reduced susceptibility to β-lactam agents due to PBP mutations.

ATCC is a trademark of American Type Culture Collection.

For details about the choice of antibiotics, the control strains, the frequency of the controls and the tables of the acceptability ranges, consult the EUCAST documents.[4,7]

11- PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of ready-to-use plates of Mueller Hinton Agar F and of the raw material used for the production of prepared plates (dehydrated Mueller Hinton Agar II REF 401740, supplemented with defibrinated horse blood and β-NAD) are tested by Antimicrobial Sensitivity Testing and for productivity properties, by comparing the results with a previously approved Reference Batch.
Productivity is tested by semi-quantitative ecometric technique with the following target strains: *S.pyogenes* ATCC 19615*, S.pneumoniae* ATCC 6305 and *L.monocytogenes* ATCC 19111. After aerobic incubation at 35-37°C for 18-24 hours, the amount of growth is evaluated and recorded. All strains must show a good growth. AST is performed according to EUCAST procedure[4] with the following strains and antimicrobial disks: *S.pneumoniae* ATCC 49619 (CLI, ERY, LEV, SXT, VAN)*, H.influenzae* ATCC 49766 (AMP, CTX, CIP, IMI, TET, SXT). After incubation at 35 ± 1°C in 4-6% CO_2 in air for 18 ± 2 hours, the inhibition zones are measured, recorded and evaluated to be within the quality control ranges reported by EUCAST.[5] Concentration of Ca^{++} and Mg^{++} are measured for all production batches of dehydrated raw material Mueller Hinton Agar II, to assure batch-to-batch reproducibility.

12 - LIMITATIONS OF THE METHOD

- EUCAST has evaluated the disk potency of 16 strategically important antibiotic disks from nine manufacturers of disks for antimicrobial susceptibility testing. The study disclosed some good and some poor quality among disks and manufacturers. It is the responsibility of laboratories to perform quality control to guarantee that the material used performs to the standards of the laboratory and the health care system.[8]
- Incorrect inoculum concentration, improper storage of antimicrobial disks, improper storage of the plates resulting in an agar depth and pH out of the specifications, excessive moisture, improper measurement of endpoints, may produce incorrect results.[9] Therefore, strict adherence to protocol is required to ensure reliable results.
- Despite the presence of animal blood and NAD, some fastidious strains may not grow or grow lightly on the medium.
- Consult the EUCAST papers for the details of disk diffusion methodology, reading and interpretations of inhibition zones, warnings, guidance documents in susceptibility testing, guidelines for detection of resistance mechanisms, clinical breakpoints.

- Mueller Hinton Agar F can be used for determination of Minimum Inhibiting Concentrations (MICs) with strips containing antimicrobial gradients. To perform this method, it is required to follow the instructions for use of the supplier of strips and to validate the work procedure in the laboratory.
- This culture medium is intended as an aid in the treatment of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- Mueller Hinton Agar F is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Mueller Hinton Agar F is not classified as dangerous according to current European legislation.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that these products do not contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use plates be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the plates inoculated with samples or microbial strains in accordance with current local legislation.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

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4. The European Committee on Antimicrobial Susceptibility Testing. EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing - Version 8.0 (January 2020). http://www.eucast.org.
5. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0, 2020. http://www.eucast.org.
6. Jonasson E, Matuschek E, Kahlmeter G. The EUCAST rapid disc diffusion method for antimicrobial susceptibility testing directly from positive blood culture bottles. J Antimicrob Chemother 2020; 75: 968–978
7. The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 10.0, 2020. http://www.eucast.org."
8. Åhman J, Matuschek E, Kahlmeter G. The quality of antimicrobial discs from nine manufacturers EUCAST evaluations in 2014 and 2017. Clinical Microbiology and Infection 2019; 25:346-352
9. Matushek E. EUCAST Educational Workshop. Technical problems and controversies in antimicrobial susceptibility testing. ECCMID 2017, Vienna, Austria.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 0	NA	2020/08
Revision 1	Removal of obsolete classification	2023/04

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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