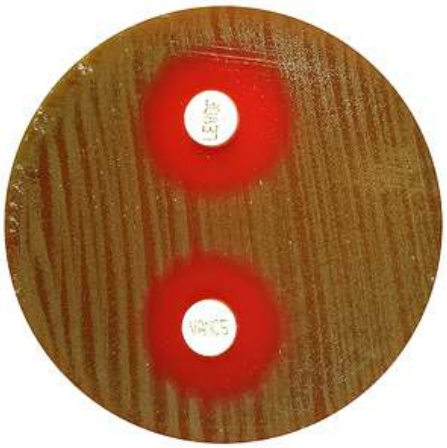
INSTRUCTIONS FOR USE

MUELLER HINTON AGAR BLOOD SHEEP

Ready-to-use plates



MHA Blood Sheep:
S.pneumoniae / levofloxacin and vancomycin

1 - INTENDED USE

In vitro diagnostic device. Culture medium for Antimicrobial Susceptibility Testing (AST) by disk diffusion method of streptococci and *Neisseria meningitidis*, isolated from clinical specimens, according to CLSI.

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 sheep blood	50.0 mL
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 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 sheep blood is recommended and standardized by CLSI[3] for testing the following fastidious organisms: *Streptococcus pneumoniae, Streptococcus* spp. β-haemolytic group, *Streptococcus* spp. *viridans* group, *Neisseria meningitidis*. Defibrinated sheep blood enables 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 Blood Sheep	Ready-to-use plates	541743	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box
Mueller Hinton Agar Blood Sheep – 150 mm	Ready-to-use plates	501743P	5 plates ø 150 mm primary packaging: 1 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, CO_2 atmosphere generators and jars, antimicrobial susceptibility paper discs.

7 - SPECIMENS

AST by disk diffusion method is designed to be used with pure culture of strains isolated from clinical specimens. Mueller Hinton Agar Blood Sheep 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.

8- TEST PROCEDURE

- 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. Do not over-dry plates.
- **Streptococci**: prepare the inoculum using colonies from an overnight (18 to 20 hours) culture on a blood agar plate. 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.
- ***N.meningitidis***: prepare the inoculum using colonies from an overnight (18 to 20 hours) culture on a chocolate agar plate incubated at 35°C with 5% CO_2. 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. Colonies grown on sheep blood agar may be used for inoculum preparation. However, the 0.5 McFarland suspension obtained from sheep blood agar will contain approximately 50% fewer CFU/mL.

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- Dip a sterile cotton swab into the suspension. 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. With streptococci test a maximum of 9 disks on a 140 mm plates and 4 disks on a 90 mm plate. With *N.meningitidis* test a maximum of 5 disks on a 140 mm plates and 2 disks on a 90 mm plate.
- 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 ± 2°C, 5% CO_2 for 20-24 hours.

9 - READING AND INTERPRETATION

Measure the diameter of zones of complete inhibition, considering the area showing no obvious, visible growth that can be detected with the unaided eye, including the diameter of the disk. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.[3]
For haemolytic streptococci, do not measure the zone of inhibition of haemolysis.
With trimethoprim and sulphonamides, antagonists in the medium allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.[3]
For specific reading instructions consult the CLSI document.[3]
Interpret zone diameters into susceptibility categories according to the current breakpoint tables.[3]

10 - USER QUALITY CONTROL

All manufactured lots of Mueller Hinton Agar Blood Sheep plates are released for sale after the Quality Control has been performed to check the compliance with the specifications, according to CLSI rules[3]. However, it is responsibility of the end-user to perform Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Select the quality control strain specified by CLSI and reported here below, to monitor the performance of the test.
Streptococcus pneumoniae ATCC 49619
For the details about the suggested QC frequency, the choice of antibiotics and the acceptability ranges, consult the CLSI document.[3]

ATCC is a trademark of American Type Culture Collection.

11- PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of ready-to-use plates of Mueller Hinton Agar Blood Sheep and of the raw material used for the production of prepared plates (dehydrated Mueller Hinton Agar II REF 401740, supplemented with defibrinated sheep blood) are tested by Antimicrobial Sensitivity Testing and for productivity properties, by comparing the results with a previously approved Reference Batch.
Productivity of Mueller Hinton Agar II REF 401740, supplemented with defibrinated sheep blood 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 CLSI procedure[3] with *S.pneumoniae* ATCC 49619 and the following disks: oxacillin 1 µg, erythromycin 15 µg, ampicillin 10 µg, cefotaxime 5 µg , ertapenem 10 µg, trimethoprim-sulfamethoxazole 25 µg . After incubation at 35 ± 1°C, 5% CO_2, for 20-24 hours, the inhibition zones are measured, recorded and evaluated to be within the quality control ranges reported by CLSI.[3]
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

- With trimethoprim and sulphonamides, antagonists in the medium may allow some slight growth; therefore, read the end point at the concentration in which there is ≥80% reduction in growth.[3]
- Amoxicillin, ampicillin, cefepime, cefotaxime, ceftriaxone, ertapenem, imipenem and meropenem may be used to treat pneumococcal infections; however, reliable disk diffusion tests with these agents do not yet exists. Their *in vitro* activity is best determined using a MIC method.[3]
- For *S.pneumoniae* isolated from CSF, penicillin and cefotaxime, ceftriazone, or meropenem should be tested by a reliable MIC method and reported routinely. Such isolates can be tested against vancomycin using the MIC or disk diffusion method.[3]
- 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.[6]
- Incorrect inoculum concentration, improper storage of antimicrobial discs, 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.[7] Therefore, strict adherence to protocol is required to ensure reliable results.
- CLSI specifies the applicability of interpretative breakpoints for *Streptococcus* spp. β-haemolytic group: large colony forming pyogenic strains streptococci with group A (*S.pyogenes*), C or G antigens, and strains with group B antigen (*S.agalactiae*).[3]
- CLSI specifies the applicability of interpretative breakpoints for *Streptococcus* spp. viridans group: *mutans* group, *salivarius* group, bovis group, *anginosus* group (previously "*S.milleri* group") and *mitis* group.[3]
- CLSI recommends the use of MH-F as an alternative to Mueller Hinton Blood Sheep for AST with *S.pneumoniae*.[3]
- Despite the presence of animal blood, some fastidious strains may not grow or grow lightly on the medium.
- Consult the CLSI papers for the details of disc 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 Blood Sheep can be used for determination of 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.

- Informational supplements to CLSI document M100, or revised versions, are periodically published, containing revised tables of antimicrobial discs and interpretative standards. The latest tables should be consulted for current recommendations.
- 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 Blood Sheep 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 Blood Sheep 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.
- Perform all AST on *N.meningitidis* in a BSC. Manipulating *N.meningitidis* outside a BSC is associated with increased risk for contracting meningococcal disease.[3]
- 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

1. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society for Microbiology (ASM), December 8, 2009.
2. Bauer AW, Perry DM, Kirby WM. Single disk antibiotic sensitivity testing of staphylococci. Analysis of technique and results. Arch Intern Med 1959; 104:208
3. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2020.
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. 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
7. 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	Σ 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 1	Updated layout and content in compliance with IVDR 2017/746	2020/10
Revision 2	Removal of obsolete classification	2023/03

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

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