INSTRUCTIONS FOR USE

HAEMOPHILUS TEST MEDIUM

Ready-to-use plates

Haemophilus Test Medium:
AST of *H.influenzae*

1 - INTENDED USE

In vitro diagnostic device. Culture medium for Antimicrobial Susceptibility Testing (AST) by disk diffusion method of clinical isolates of *Haemophilus* species, according to the Clinical and Laboratory Standards Institute (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
Yeast Extract	5.0 g
NAD	15.0 mg
Bovine haematin	15.0 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 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 yeast extract, NAD and haematin (Haemophilus Test Medium-HTM), as growth-promoting additives, has been proposed by Jorgensen *et al.*[6] in 1987 and is currently recommended by CLSI[3] for testing *Haemophilus* spp. CLSI document M100[4] reports the zone diameter breakpoints and interpretative categories for *H.influenzae* and *H.parainfluenzae*; testing and reporting recommendations for other species of *Haemophilus* are included in the CLSI document M45.[7] Disk diffusion tests performed with HTM allows accurate categorization of susceptible and resistant strains and is easier to interpret than tests performed with Mueller-Hinton chocolate agar.[6]

4 - PHYSICAL CHARACTERISTICS

Medium appearance	beige-amber, limpid
Final pH at 20-25 °C	7.3 ± 0.1

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Haemophilus Test Medium	Ready-to-use plates	549901	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, CO_2 atmosphere generators and jars, antimicrobial susceptibility paper discs.

7 - SPECIMENS

AST by disk diffusion method is designed to be used with pure cultures of strains isolated from clinical specimens. Haemophilus Test Medium is not intended for microbial isolation directly from clinical specimens.

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.
- Prepare the inoculum using colonies from an overnight (20 to 24 hours) culture on a chocolate 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. Colonies grown on sheep blood agar may be used for inoculum preparation. The 0.5 McFarland suspensions contain approximately 1 to 4 x 10^8 CFU/mL. Use care in preparing this suspension because higher inoculum concentrations may lead in false-resistant results with some β-lactam antimicrobial agents, particularly when β-lactamase producing strains of *H.influenzae* are tested.
- 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.

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- 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. Test a maximum of 9 disks on a 140 mm plates and 4 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 16-18 hours.

9 - READING AND INTERPRETATION

Measure the diameter of zones of complete inhibition as judged by the unaided eye, including the diameter of the disk. Hold the plates a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.
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.
Interpret zone diameters into susceptibility categories according to the current breakpoint tables reported by CLSI M100[3] for *H.influenzae* and *H.parainfluenzae* and by CLSI M45 for other species of *Haemopilus*.[7]

10 - USER QUALITY CONTROL

All manufactured lots of Haemophilus Test Medium 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, 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 strain specified by CLSI (*H.influenzae* ATCC 49247) to monitor the performance of the test.
For the details about the suggested QC frequency, the choice of antibiotics and the acceptability ranges, consult the CLSI document.[3]

11- PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of ready-to-use plates of Haemophilus Test Medium is tested by Antimicrobial Sensitivity Testing, according to CLSI procedure[3] with *H.influenzae* ATCC 49247 and the following disks: tetracycline 30 µg, imipenem 10 µg, ampicillin 10 µg, cefotaxime 30 µg, trimethoprim-sulfamethoxazole 25 µg. After incubation at 35-37°C, 5% CO_2, for 18-24 hours, the inhibition zones are measured, recorded and evaluated to be within the quality control ranges reported by CLSI.[3]
Concentrations of Ca^{++} and Mg^{++} are measured for all production batches of dehydrated raw material Mueller Hinton Agar II (REF 401740), to assure batch-to-batch reproducibility.

12 - LIMITATIONS OF THE METHOD

- With trimethoprim and sulphonamides, antagonists in the medium my allow some slight growth; therefore, read the end point at the concentration in which there is ≥80% reduction in growth compared with the control.[3]
- For isolates of *H.influenzae* from CSF, only results of testing with ampicillin, any of 3rd-generation cephalosporins listed below, chloramphenicol and meropenem are appropriate to report.[3]
- Amoxicillin-clavulanate, azithromycin, cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime and clarithromycin are used as empiric therapy for respiratory tract infections due to *Haemophilus* spp. The results of susceptibility tests with these antimicrobial agents are often not necessary for management of individual patients.[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.[8]
- 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.[9] Therefore, strict adherence to protocol is required to ensure reliable results.
- 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.
- 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

- Haemophilus Test Medium 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.
- Haemophilus Test Medium 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

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. Jorgensen JH, Redding JS, Maher LA, Howell AW. Improved medium for antimicrobial susceptibility testing of Haemophilus influenza. J Clin Microbiol 1987; 25:2105- 2113.
7. Clinical and Laboratory Standards Institute (CLSI). Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. 3rd ed. CLSI guideline M45. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2016.
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 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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