microgen
Ready Prepared Media

Technical Information

Haemophilus Test Agar Plate

Product Code: PM 2259

Application: Recommended for the susceptibility testing of Haemophilus influenzae .

Composition**

Ingredients	Gms / Litre
HM infusion B from#	300.000
Acicase ##	17.500
Yeast extract	5.000
Starch	1.500
Agar	17.000
Haematin Growth Supplement (MS2117)	**1 Vial**
NAD (2x7.50 mg)	15.000mg
Haematin (2x7.50 mg)	15.000mg
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef infusion from,

##-Equivalent to casein acid hydrolysate

Principle & Interpretation

Haemophilus species are nutritionally fastidious in nature. They require either exogenous hemin (X-factor) or NAD (V-factor) or both (1). Due to this reason, Mueller Hinton Agar, which is used for antimicrobial susceptibility of bacteria (2,3,4), cant be used for the antimicrobial susceptibility testing of *Haemophilus*. Haemophilus Test Agar Base, studied by Jorgensen et al (5,6) is used for the susceptibility testing of *Haemophilus influenzae*. This medium has similar composition as Mueller Hinton Agar, with the addition of yeast extract and added growth supplements. Haemophilus Test Agar Base is simple, transparent and posses minimum risk of antagonism of antimicrobial agents (5). Haemophilus Test Agar Base is also recommended by the United States National Committee for Clinical Laboratory Standards (NCCLS) for both dilution and disc diffusion assays (7). This medium scores over Mueller Hinton Agar with heamoglobin over clarity, thereby enabling proper visualization of inhibition zones. It also has low levels of the nucleotide thymidine, which allows testing of trimethoprim / sulphamethoxazole.

Haemophilus Test Agar Base contains HM infusion B from and acicase, which provide essential nutrients to the organisms. Yeast extract serves as a source of B complex vitamins. Starch acts as a protective colloid against toxic substances presentin the medium. The surface of a Haemophilus Test Agar Base with added nutrients is inoculated either by using swab or by spreading the suspension. Antimicrobial discs i.e. paper discs impregnated with specific amount of antibiotics or other antimicrobial agents are placed on the surface of medium spaced properly. The plates are incubated in a CO_2incubator and subsequently the inhibition zones around each disc are read. Comparing the zones of inhibition with the NCCLS standards, the determination as to whether the organism is susceptible, resistant or intermediate in its response to the antimicrobial substances is made (7).

Type of specimen

Isolated Microorganism from clinical samples.

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Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,5).After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. The plates are to be incubated at 5-7% carbon dioxide at 35-37°C for 18-24 hours for appropriate results.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
3. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored atrecommended temperature.

Methodology

Either streak, inoculate or surface spread the test inoculum (50-100 cfu) aseptically on the plate .

Quality Control

Appearance
Sterile Haemophilus Test Agar in 90mm disposable plate with smooth surface and absence of black particles/cracks/bubbles
Colour
Light amber coloured medium
Quantity of medium
25 ml of medium in 90 mm plate
pH
6.80 - 7.20
Sterility Check
Passes release criteria
Cultural Response
Cultural characteristics observed in 5-7% carbon dioxide after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum(CFU)	Growth	Recovery
Haemophilus influenzaeATCC 49766	50-100	luxuriant	>=70%
Enterococcus faecalisATCC 29212 (00187*)	50-100	good- luxuriant	>=70%
Streptococcus pyogenesATCC 19615	50-100	good- luxuriant	>=70%
Neisseria meningitidisATCC 13090	50-100	good-luxuriant	>=70%
Staphylococcus aureussubsp.aureus ATCC 25923 (00034*)	50-100	good- luxuriant	>=70%

(*) - Corresponding WDCM numbers

Storage and Shelf Life

- On receipt store between 2-8°C Use before expiry date on the label.
- Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with samplemust be decontaminated and disposed of in accordance with current laboratory techniques (3,5).

Further Reading

1. Barry A. L., Garcia F., and Thrupp L. D., 1970, Am. J. Clin. Pathol., 53 :149.
2. Bauer A. W., Kirby W. M., Sherris J. C. and Turck M., 1966, Am. J. Clin. Pathol. 45:493.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen J. H., Redding J. S., Maher L. A. and Howell A. W., 1987, J. Clin. Microbiol., 25:2105.
5. Jorgensen J. H., Howell A. W., and Maher L. A., J. Clin. Microbiol, 28:985.
6. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
7. Ryan K. J., Schoenknecht F. D., and Kirby W. M., 1970, Hospital Practice, 5:91.

Disclaimer

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate
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