Haemophilus Test Agar Base

Intended Use

Recommended for the susceptibility testing of Haemophilus influenzae.

Typical Composition (g/litre)

HM infusion B 300.0 ;Yeast extract 5.0 ; Acicase 17.50 ; Starch 1.50 ; Agar 17.0

Mode of Action

Haemophilus Test Agar Base contains HM infusion B from and Acicas, which provide nitrogeneous and carbonaceous compounds, long chain amino acids and essential nutrients to the organisms. Yeast extract serves as a source of B complex vitamins. Starch acts as a protective colloid against toxic substances present in the medium.

Preparation

Suspend 21.5 grams in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add the rehydrated contents of 1 vial of Haemophilus Growth Supplement. Mix well and pour into sterile Petri plates.

Final pH (at 25°C) 7.4±0.2

Storage

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Specimen

Isolated Microorganism from clinical samples.

Experimental Procedure and Evaluation

Haemophilus Test Agar Base, studied by Jorgensen et al 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. Haemophilus Test Agar Base is also recommended by (NCCLS) for both dilution and disc diffusion assays. 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.

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 CO2 incubator 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.

Quality Control

Organism	Inoculum	Growth	Recovery
Haemophilus influenzae ATCC 49766	50 - 100	Luxuriant	≥70 %
Enterococcus faecalis ATCC 00087	50 - 100	Good-Luxuriant	≥70 %
Streptococcus pyogenes ATCC 19615	50 - 100	Good-Luxuriant	≥70 %
Neisseria meningitides ATCC 13090	50 - 100	Good-Luxuriant	≥70 %
Staphylococcus aureus ATCC 25923	50 - 100	Good-Luxuriant	≥70 %

Reference

1. Jorgensen J. H., Redding J. S., Maher L. A. and Howell A. W., 1987, J. Clin. Microbiol.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. 2. Bauer A. W., Kirby W. M., Sherris J. C. and Turck M., 1966, Am. J. Clin. Pathol.
5. 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.
6. Ryan K. J., Schoenknecht F. D., and Kirby W. M., 1970, Hospital Practice.