Haemophilus Test Agar Base

M1259

Haemophilus Test Agar Base with added growth supplement is recommended for the susceptibility testing of *Haemophilus influenzae* .

Composition**

Ingredients	Gms / Litre
Beef infusion from	300.000
Casein acid hydrolysate	17.500
Yeast extract	5.000
Starch	1.500
Agar	17.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 21.5 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add the rehydrated contents of 1 vial of Haemophilus Growth Supplement (FD117). Mix well and pour into sterile Petri plates.

Principle And 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 (2-4), cant be used for the antimicrobial susceptibility testing of *Haemophilus* . Also, addition of blood to Mueller Hinton Agar to supply the essential growth nutrients makes the medium opaque, rendering it unsuitable for antimicrobial susceptibility testing.

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 beef infusion and casein acid hydrolysate, 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 present in 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 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 (7).

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.7% Agar gel

Colour and Clarity of prepared medium

Light amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.3% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
M1259: Cultural characteristics observed with added Haemophilus Growth Supplement (FD117) in 5-7% carbon dioxide after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
Haemophilus influenzae ATCC 49766	50-100	luxuriant	>=70%
Enterococcus faecalis ATCC 29212	50-100	good-luxuriant	>=70%
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant	>=70%
Neisseria meningitidis ATC 13090	50-100	good-luxuriant	>=70%
Staphylococcus aureus ATCC 25923	50-100	good-luxuriant	>=70%

Storage and Shelf Life
Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference
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4.Barry A. L., Garcia F., and Thrupp L. D., 1970, Am. J. Clin. Pathol., 53 :149.
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Revision : 1 / 2011

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