Haemophilus Test Agar Base (DM1273)

Intended Use
Haemophilus Test Agar Base (DM1273) is recommended for the susceptibility testing of *Haemophilus influenzae*.

Product Summary and Explanation
Haemophilus species are nutritionally fastidious in nature requiring either exogenous hemin (X-factor) or NAD (Vfactor) or both.[1] Haemophilus influenzae require complex media for growth. These complex media have aggravated the routine susceptibility testing of *H. influenzae* because of antagonism between some essential nutrients and certain antimicrobial agents. In 1966, Bauer, Kirby and others developed a standardized procedure for the antimicrobial susceptibility testing of common, rapidly growing bacteria in which Mueller Hinton Agar was selected as the test medium. This medium is not satisfactory for fastidious organisms such as some streptococci, gonococci and *Haemophilus* species.[2-4] 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] has been specifically formulated 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] A major advantage of HTM Agar compared with Mueller Hinton Chocolate Agar is optical clarity, permitting zone diameter measurements from the bottom of the dish as is the standard test procedure for non-fastidious organisms on Mueller Hinton Agar. Comparisons with Mueller-Hinton Chocolate Agar have shown an overall agreement of 99.6%.[8] Furthermore, HTM Agar contains low levels of thymidine and is, therefore, suitable for testing trimethoprim/sulfamethoxazole.

Principles of the Procedure
Haemophilus Test Agar Base contains beef infusion and casein acid hydrolysate, which provides carbon, nitrogen and oth essential nutrients required for growth of organisms. Yeast extract serves as a source of B complex vitamins. Starch acts as a protective colloid against toxic substances present in the medium.

Formula / Liter

Ingredients	Gms / Liter
Beef infusion from	300.00
Casein acid hydrolysate	17.50
Yeast extract	5.00
Starch	1.50
Agar	17.00
Final pH: 7.4 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 21.5 grams of the medium in 500 ml of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 50°C and aseptically add the rehydrated contents of 1 vial of Haemophilus Growth Supplement (MS085).
5. Mix well and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light amber coloured, clear to slightly opalescent gel forms in Petri plates
Reaction of 4.3% solution	pH 7.4 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.7% Agar gel

Expected Cultural Response: Cultural characteristics observed with added Haemophilus Growth Supplement (MS085) in 5-7% carbon dioxide after an incubation at 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	*Haemophilus influenza ATCC 49766*	50-100	good-luxuriant	>=70%
2.	*Enterococcus faecalis ATCC 29212*	50-100	good-luxuriant	>=70%
3.	*Streptococcus pyogenes ATCC 19615*	50-100	good-luxuriant	>=70%
4.	*Neisseria meningitidis ATCC 13090*	50-100	good-luxuriant	>=70%
5.	*Staphylococcus aureus ATCC 25923*	50-100	good-luxuriant	>=70%

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Prepare a Gram stain before starting susceptibility testing to confirm culture purity and to confirm tentative identification of *Haemophilus*.
2. Use several well-isolated colonies taken directly from an overnight (preferably 20-24 hours) Chocolate Agar plate (DM314) as the source of the inoculum.
3. A rapid ß-lactamase test should be utilized for rapid detection of strains that are resistant to penicillin, ampicillin or amoxicillin.
4. Prepare a suspension of the test organism in Mueller Hinton Broth (DM173) or 0.9% saline. This suspension should be adjusted to the turbidity of the 0.5 McFarland standard using a photometric device. This suspension will contain approximately 1-4 × 10 CFU/mL. Care must be exercised in preparing this suspension because higher inoculum concentrations may lead to false-resistant results with some ß-lactam antibiotics, particularly when ß-lactamase-producing strains of *H. influenzae* are tested.
5. Consult the product literature or the CLSI Approved Standard M2[9] for details on plate inoculation and use of antimicrobial discs.

Bauer-Kirby procedure:

1. This procedure is based on the diffusion through an agar gel of antimicrobial substances which are impregnated on paper discs.
2. Earlier methods made use of discs from high and low antimicrobial concentrations and the presence or absence of inhibition zones for their interpretation, in this method discs with single concentration of antimicrobial agent and zone diameters are correlated with minimal inhibitory concentrations (MICs).
3. In the test procedure, a standardized suspension of the organism is swabbed over the entire surface of the medium. Paper discs impregnated with specified amounts of antibiotic or other antimicrobial agents are then placed on the surface of the medium, the plate is incubated and zones of the inhibition around each disc are measured.
4. The determination as to whether the organism is susceptible, resistant or intermediate in its response to the agent is made by comparing zone diameters obtained to those provided with CLSI document M2.[9]
5. Various factors have been identified as influencing disc diffusion susceptibility tests. These include the medium, agar depth, disc potency, inoculums concentration, pH and beta-lactamase production by test organisms.[9-12]

Results

1. Examine the plates after 16-18 hours of incubation. A confluent "lawn" of growth should be obtained. If only isolated colonies grow, the inoculum was too light and the rest should be repeated.
2. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disc, to the nearest whole millimeter, using calipers, a ruler, or a template prepared for this purpose. The measuring device is held on the back of the plate, which is held over a black, non-reflecting background and illuminated from above. The endpoint should be taken as the area showing no obvious visible growth that can be

detected with the unaided eye. Disregard faint growth of tiny colonies which can be detected with difficulty near the edge of the obvious zone of the inhibition.

3. Consult the product literature or the CLSI Approved Standard M2[(9)] for details on interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Haemophilus Test Agar Base
Product Code : DM1273
Available Pack sizes : 500gm

References

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Further Information

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