Technical Information

Mueller Hinton Agar No. 2

Product Code: DM 2084

Application: - Mueller Hinton Agar No.2 is used for testing susceptibility of common and rapidly growing bacteria using antimicrobial discs by the Bauer - Kirby method. Manufactured to contain low levels of thymine, thymidine, calcium and magnesium.

Composition**

Ingredients	Gms / Litre
HM infusion solids B #	2.000
Acicase ##	17.500
Starch	1.500
Agar	17.000
Final pH (at 25°C)	7.4±0.1

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef heart infusion

- Equivalent to Casein acid hydrolysate

Principle & Interpretation

The goal of susceptibility test is to predict through an in vitro assessment the likelihood of successfully treating a patient's infection with a particular antimicrobial agent (1). The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species (2). Other media were subsequently developed that replaced the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria* species, but it became widely used in the determination of sulfonamide resistance of gonococci and other organisms. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing (3). Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in NCCLS (National Committee for Clinical Laboratory Standards), now CLSI (Clinical and Laboratory Standards Institute) Approved Standard (4). Mueller Hinton Agar has been selected by the CLSI for several reasons: i. It demonstrates good batch-to-batch reproducibility for susceptible testing. ii. It is low in sulfonamide, trimethoprim and tetracycline inhibitors. iii. It supports the growth of most non-fastidious bacterial pathogens and iv. Many data and much experience regarding its performance have been recorded (1). Mueller Hinton Agar No. 2 is used in the susceptibility testing of rapidly growing aerobic and facultatively anaerobic bacteria from clinical specimens. Kirby-Bauer et al recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration (5). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (6). The medium is designed to give a low thymine and thymidine content and also the calcium and magnesium ion concentration is adjusted as recommended by CLSI (3). The medium is not recommended for fastidious organisms. Thymine and thymidine inhibit sulfonamide and trimethoprim (9,10) activity and calcium and magnesium (11,12) interferes with the activity of aminoglycoside antibiotics. HM infusion solids B and acicase supply nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch serves as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which acts as a source of energy. These ingredients are selected for low thymine and thymidine content as determined by MIC values for *Enterococcus faecalis* with sulfamethoxazole trimethoprim (SXT). Calcium and magnesium ion concentrations are adjusted to provide the amounts recommended by CLSI to give the correct MIC values with aminoglycosides and *Pseudomonas aeruginosa* (3) The Kirby-Bauer procedure is based on agar diffusion of antimicrobial substances impregnated on paper discs.

This method employs disc with a single concentration of antimicrobial agent and the zone diameters observed are correlated with minimum inhibitory concentration (MIC) values (2, 3, 7). A standardized suspension of the organism is swabbed over the entire surface of the medium. Paper discs impregnated with specific amounts of antimicrobial agents are then placed on the surface of the medium, incubated and zones of inhibition around each disc are measured. The susceptibility is determined by comparing with CLSI standards (8). The various factors, which influence disc diffusion susceptibility tests, are agar depth, disc potency, inoculum concentration, pH of the medium and beta-lactamase production by test organisms (1, 8).

Type of specimen

Clinical samples: Pure cultures isolated from urine, stool, blood etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (13, 14).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic 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

This medium is recommended for susceptibility testing of pure cultures only. Inoculum density may effect the zone size. Heavy inoculum may result in smaller zones or too less inoculum may result in bigger zones. Fastidious organisms may not grow on this medium and may require supplementation of blood. Fastidious anaerobes may not grow on this medium. As antimicrobial susceptibility is carried with antibiotic disc, proper storage of the disc is desired which may effect the potency of the disc. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo

Performance and Evaluation

Performace of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Methodology

Suspend 38.0 grams of dehydrated powder media in 1000 ml distilled water. Mix thoroughly & 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. Shake well before pour into sterile Petri plates.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.7% agar gel.

Colour and Clarity

Light amber coloured clear to slightly opalescent gel froms in Petri plates

Reaction

Reaction of 3.8% w/v aqueous solution at 25°C. pH : 7.3±0.1

pH Range

7.20-7.40

Cultural Response

DM 2084: Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=70%
Haemophilus influenzae ATCC 49247	50-100	good- luxuriant(on Mueller Hinton Chocolate Agar)	>=70%
Neisseria gonorrhoeae ATCC 49226	50-100	luxuriant	>=70%
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=70%
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	luxuriant	>=70%
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=70%
Streptococcus pneumoniae ATCC 6305	50-100	luxuriant (on Mueller Hinton Blood Agar)	>=70%

Key: *Corresponding WDCM numbers.

Storage and Shelf Life

Dried Media: Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (13, 14).

Further Reading

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Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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