Mueller Hinton Agar No. 2

**Intended Use:**
Recommended 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**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM infusion solids B</td>
<td>2.000</td>
</tr>
<tr>
<td>Acicase ##</td>
<td>17.500</td>
</tr>
<tr>
<td>Starch</td>
<td>1.500</td>
</tr>
<tr>
<td>Agar</td>
<td>17.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.3±0.1</td>
</tr>
</tbody>
</table>

**Directions**

Suspend 38.0 grams in 1000 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. Mix well and pour into sterile Petri plates.

**Principle And 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 (9). The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species (8). 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 (12). 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 (10). 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 (9). 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 (1). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (13). 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 (12). The medium is not recommended for fastidious organisms. Thymine and thymidine inhibit sulfonamide and trimethoprim (7,4) activity and calcium and magnesium (14,2) interferes with the activity of aminoglycoside antibiotics. HM infusion solids B and acicase provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch acts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which serves 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* (12) 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 (8, 12, 3). 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 (11). 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 (9,11).

**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 (5,6).
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**
1. This medium is recommended for susceptibility testing of pure cultures only.
2. Inoculum density may affect the zone size. Heavy inoculum may result in smaller zones or too less inoculum may result in bigger zones.
3. Fastidious organisms may not grow on this medium and may require supplementation of blood.
4. Fastidious anaerobes may not grow on this medium.
5. As antimicrobial susceptibility is carried with antibiotic disc, proper storage of the disc is desired which may affect the potency of the disc.
6. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.

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

**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 froms in Petri plates

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

**pH**
7.20-7.40

**Cultural Response**
Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><em>Haemophillus influenzae</em> ATCC 49247</td>
<td>50-100</td>
<td>good-luxuriant( on Mueller Hinton Chocolate Agar)</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em> ATCC 49226</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Minimum Growth</th>
<th>Morphology</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853(00025*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp. aureus ATCC 25923 (00034*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212 (00087*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em> ATCC 6305</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
</tbody>
</table>

Key: (*) Corresponding WDCM numbers.

**Storage and Shelf Life**

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 in order 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 (5,6).

**References**

In vitro diagnostic medical device

CE Marking

Storage temperature

10°C - 30°C

Do not use if package is damaged

HiMedia Laboratories Pvt. Limited,
23 Vadhani Industrial Estate,
LBS Marg, Mumbai-86, MS, India

CE Partner 4U, Esdoornlaan 13, 3951 DB Maarn The Netherlands,
www.cepartner4u.eu

Disclaimer:
User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.