Mueller Hinton Agar plate w/ 5% Sheep Blood

Intended Use:
Recommended for determination of susceptibility of *Streptococcus* species to antimicrobial agents.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM infusion from #</td>
<td>300.000</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>Sheep Blood</td>
<td>50.000 ml</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters**

# Equivalent to Beef, infusion from

## Equivalent to Casein acid hydrolysate

**Directions**

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

**Principle And Interpretation**

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species (5). It is further enriched by the addition of sterile blood. Traditionally blood agar bases have incorporated either casein hydrolysate to give rapid production of large colonies or beef infusion to give defined hemolytic reactions. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing (9). Mueller Hinton Agar is used as a base for preparing media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives. It supports rapid and luxuriant growth of fastidious and nonfastidious organisms. Also, this medium promotes typical colonial morphology; better pigment production and more sharply defined haemolytic reactions. Mueller Hinton Agar with 5% sheep blood is recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae*.

Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard (10).

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 (11). HM infusion 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 sulfamethoxazoletrimethoprim (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* (9). 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 (2,5,9). 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 (6,8).

**Type of specimen**

Clinical samples: Isolated Microorganisms from urine, stool, blood etc.

**Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,10). After use, contaminated materials must be sterilized by autoclaving before discarding.
Warning and Precaution
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. As antimicrobial susceptibility is carried with antibiotic disc, proper storage of the disc is desired which may affect the potency of the disc.
4. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.
5. Each lot of the medium has been tested for the organisms specified on the COA.

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
Sterile Mueller Hinton Agar + 5% Sheep Blood in 90 mm disposable plates.

Colour
Red coloured medium

Quantity of medium
25ml of medium in disposable plate

Reaction
7.10-7.50

Sterility test
Passes release criteria

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours with added 5% w/v sterile sheep blood.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth</th>
<th>Recovery</th>
<th>Haemolysis</th>
<th>Clindamycin</th>
<th>Erythromycin</th>
<th>Vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>luxuriant</td>
<td>&gt;=70%</td>
<td>beta</td>
<td>CD 2 mcg</td>
<td>E 15 mcg</td>
<td>VA 30 mcg</td>
</tr>
<tr>
<td>ATCC 19615</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>luxuriant</td>
<td>&gt;=70%</td>
<td>alpha</td>
<td>19 -25 mm</td>
<td>25 -30 mm</td>
<td>20 -28 mm</td>
</tr>
<tr>
<td>ATCC 49619</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Storage and Shelf Life
Store between 2-8°C. Use before expiry date on the label.

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 (3,4).

Reference

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.