HiEncap™ Mueller Hinton Agar No. 2 contains low levels of thymine, thymidine, calcium and magnesium and used for testing susceptibility of common and rapidly growing bacteria using antimicrobial discs by the Bauer - Kirby method.

**Composition**

**Ingredients**
- Casein acid hydrolysate: 17.500 Gms
- Beef heart infusion: 2.000 Gms
- Starch, soluble: 1.500 Gms
- Agar: 17.000 Gms
- Final pH (at 25°C): 7.3±0.1

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

**Directions**

Each capsule contains 9.5 gms of medium. Suspend 1 capsule in 250 ml (4 capsules in 1000 ml) distilled or purified water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and dispense as desired.

**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 (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. Beef heart infusion and casein acid hydrolysate 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 (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).

**Quality Control**

**Appearance**
Gelatin capsule containing cream to yellow coloured granular media

**Gelling**
Firm, comparable with 1.7% agar gel.

**Colour and Clarity of prepared medium**
Light amber coloured clear to slight opalscent gel froms in Petri plates.

**Quantity**
Each capsule contains 9.5 grams of medium sufficient for 250 ml media

**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 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</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>&gt;=70 %</td>
</tr>
<tr>
<td><em>Haemophilus influenza</em> ATCC 49247</td>
<td>50 -100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td>(on Mueller Hinton Chocolate Agar)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em> ATCC 49226</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td>(w/5% CO2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212</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>
<tr>
<td>(on Mueller Hinton Blood Agar)</td>
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</tbody>
</table>

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

**Reference**