Diagnostic Sensitivity Test Agar (D.S.T. Agar)

**Intended Use:**
Diagnostic Sensitivity Test Agar (D.S.T. Agar) is used as an antibiotic sensitivity-testing medium for antibiotic sensitivity testing of fastidious pathogens such as *Neisseria, Streptococcus* and *Haemophilus* species with blood enrichment from clinical samples.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteose peptone</td>
<td>10.000</td>
</tr>
<tr>
<td>HMV infusion solids #</td>
<td>10.000</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>2.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3.000</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>2.000</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>1.000</td>
</tr>
<tr>
<td>Adenine sulphate</td>
<td>0.010</td>
</tr>
<tr>
<td>Guanine hydrochloride</td>
<td>0.010</td>
</tr>
<tr>
<td>Uracil</td>
<td>0.010</td>
</tr>
<tr>
<td>Xanthine</td>
<td>0.010</td>
</tr>
<tr>
<td>Aneurine</td>
<td>0.00002</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 43.04 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. For blood agar, cool the base to 45-50°C and add 7% v/v sterile defibrinated horse blood aseptically. Mix well with gentle rotation and pour into sterile Petri plates.

**Principle And Interpretation**
Diagnostic Sensitivity Test Agar is recommended for diagnostic as well as testing susceptibility of organisms to antibiotics and chemotherapeutic agents such as Sulfonamides. The latter produce well defined zones due to the absence of interfering substances.

The medium is nutritionally rich due to presence of amino acid bases and glucose. The salts present, helps in avoiding sudden pH shifts due to acid production, which might affect the susceptibility test and haemolytic reactions (2) and the MIC values of pH susceptible antimicrobials (1). Aneurine acts as vitamin source which improves the growth of several organisms especially Staphylococci. The agar used in the formulation has been specially processed to allow unimpeded diffusion of antimicrobials from discs (6). Addition of the bases like adenine, guanine, uracil and xanthine improve the antibiotic testing performance of the medium.

The reactive levels of thymidine and thymine must be sufficiently reduced to avoid antagonism of trimethoprim and sulfonamides which is an essential requirement for satisfactory antimicrobial susceptibility media. The requirement is achieved by addition of lysed horse blood to Diagnostic Sensitivity Testing medium. The level of thymidine is further reduced due to the action of thymidine phosphorylase, released from lysed horse erythrocytes (2). Thymidine-dependent organisms will not grow in absence of thymidine or will grow poorly in media containing reduced levels (7).

For less demanding organisms like Micrococci, *Salmonella, Shigella* coliform bacteria and *Proteus* species, this medium can be used without blood. For fastidious organisms like *Haemophilus influenzae, Neisseria meningitides*, alpha and beta haemolytic Streptococci blood enrichment is necessary.

Antibiotic susceptibility test is performed as follows: Suspension of test organisms is spread on the surface of the medium. Sensitivity discs (6) are equally spaced on the seeded medium surface and incubated at 37°C for 18 hours. The zones of inhibition obtained are recorded. This medium has reduced thymidine activity and this will affect its performance as a...
primary isolation medium.

**Type of specimen**
Clinical samples: Isolated samples from Blood, urine, respiratory samples, and other clinical material.

**Specimen Collection and Handling**
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5). 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. The salts present, helps in avoiding sudden pH shifts due to acid production, which might affect the susceptibility test and haemolytic reactions (2) and the MIC values of pH susceptible antimicrobials (1).
2. Some fastidious organisms may not grow.
3. Inoculum density affects inhibition zone. Heavy inoculum may result in smaller zones while scanty growth may result in enlarged zones.

**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.

**Quality Control**

**Appearance**
Cream to yellow homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**
Basal medium : Light amber coloured, clear to slightly opalescent gel forms. After addition of 7%w/v sterile defibrinated blood : Cherry red coloured, opaque gel forms in Petri plates.

**Reaction**
Reaction of 4.3% w/v aqueous solution at 25°C. pH : 7.4±0.2

**pH**
7.20-7.60

**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><em>Staphylococcus aureus subsp. aureus ATCC 25923 (00034</em>)</em>*</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><em><em>Escherichia coli ATCC 25922 (00013</em>)</em>*</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><em><em>Enterococcus faecalis ATCC 29212 (00087</em>)</em>*</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><strong>Neisseria meningitidis ATCC 13090</strong></td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><strong>Proteus mirabilis ATCC 25933</strong></td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><strong>Micrococcus luteus ATCC 10240</strong></td>
<td>50-100</td>
<td>luxuriant (with the addition of blood)</td>
<td>&gt;=70%</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
**Salmonella Typhi ATCC 6539**

Salmonella Typhi ATCC 6539 is luxuriant, with a growth of 50-100% and >=70%.

**Streptococcus pneumoniae ATCC 6305**

Streptococcus pneumoniae ATCC 6305 is luxuriant with the addition of blood, with a growth of 50-100% and >=70%.

**Streptococcus pyogenes ATCC 19615**

Streptococcus pyogenes ATCC 19615 is luxuriant with the addition of blood, with a growth of 50-100% and >=70%.

**Shigella flexneri ATCC 12022 (00126*)**

Shigella flexneri ATCC 12022 is luxuriant, with a growth of 50-100% and >=70%.

**Key:** *Corresponding WDCM numbers.

**Storage and Shelf Life**

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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 (4,5).

**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.