Isotonic Sensitivity Test Agar

DM604 A semi-defined medium for Antimicrobial Susceptibility (Sensitivity) Testing.

Typical formula* grams per litre

<table>
<thead>
<tr>
<th>Component</th>
<th>Grams per litre</th>
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</thead>
<tbody>
<tr>
<td>Peptone Mixture</td>
<td>16.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.0</td>
</tr>
<tr>
<td>Starch</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>2.8</td>
</tr>
<tr>
<td>Di-Sodium Hydrogen Phosphate</td>
<td>0.4</td>
</tr>
<tr>
<td>Sodium Glycerophosphate</td>
<td>0.22</td>
</tr>
<tr>
<td>Sodium Gluconate</td>
<td>0.2</td>
</tr>
<tr>
<td>Sodium Acetate</td>
<td>1.0</td>
</tr>
<tr>
<td>Uridine</td>
<td>0.3</td>
</tr>
<tr>
<td>Defined Chemical Mixture</td>
<td>0.078</td>
</tr>
<tr>
<td>Agar</td>
<td>12.0</td>
</tr>
</tbody>
</table>

pH approx. 7.3

Directions

1. Suspend by swirling 35.9g of powder in 1 litre of distilled or deionised water.
2. Sterilise by autoclaving at 121°C (15p.s.i.) for 15 minutes.
3. If required cool to 55°C and add 5-7% sterile lysed horse blood to enhance the growth of fastidious organisms.
4. Mix thoroughly before pouring.

Description

The Antimicrobial Susceptibility Test (AST) is widely acknowledged as being of great importance in epidemiological studies and in determining the rational use of antimicrobial agents. The function of the AST is to make an evaluation of a clinical isolate’s response to selected antimicrobial agents, and convert these findings into a prediction that will ensure a favourable clinical outcome. The detection of antimicrobial resistance, through the AST, can quickly alert a clinician to the use of any inappropriate antimicrobial therapy.

Within the clinical laboratory it is important that standard procedures are set in place and followed. Laboratories therefore perform the AST in accordance with standards set down by regulatory bodies such as the NCCLS (National Committee for Clinical Laboratory Standards), ICS (International Collaborative Study) or DIN (Deutsche Institut fur Normung). Such standards are detailed and based on exhaustive research conducted in laboratories worldwide.

A study undertaken by Ecrisson and Sherris emphasised that one of the main factors affecting the AST is the medium on which it is performed. The mineral content of the medium is particularly important when related to specific organism-antimicrobial combinations as varying levels can noticeably affect performance. Pseudomonas aeruginosa for instance, when tested with Gentamicin can give a range of Minimum Inhibitory Concentrations (the MIC being the lowest concentration of an antibiotic preventing growth) over several dilution steps, solely due to the variation in the cation content of the medium used.

Standardisation of the mineral content is therefore important and set levels must be maintained between subsequent batches. Contributing to these levels is the actual agar used, as this will contain Ca²⁺ and Mg²⁺ ions which will be released into the medium as their complexed forms dissociate on heating. The proportions of these ions can be maintained at a set level by the careful selection of the agar used and stringent monitoring through Quality Control.

Due to the fact that medium components can radically affect the outcome of the AST, the ideal characteristics of such media have been described by various workers in some detail. Such a sensitivity testing medium should have the following characteristics:

1. It must be capable of supporting the growth of the majority of pathogens for which susceptibility tests are required, without the need for further supplementation.
2. There should be batch-batch reproducibility.
3. The medium should not block, or enhance the action of the antimicrobials under test. (e.g. it should be free of components known to be antagonistic such as thymidine).
4. The pH of the medium should remain constant during tests because the activity of some antimicrobials is strongly affected by pH shift, particularly towards low pH (e.g. Erythromycin is less active in low pH conditions).
5. The medium should be approximately isotonic for bacteria and it should be appropriate for the addition of blood, when required, for the growth of fastidious organisms.
Various workers have reported on the development of chemically defined media, such as Synthetic Amino Acid Medium, which come close to the ideal, but the expense of producing these formulations makes them uneconomic for routine use. This makes Isotonic Sensitivity Test Agar a ‘best compromise’ in terms of economy and reproducibility for routine use in the AST.

Supplemental Information

A certain proportion of strains of common pathogens require nutritionally supplemented media for their growth. Various supplements can be added to Isotonic Sensitivity Test Agar to cope with these fastidious organisms (see below). However, these substances can interfere with the activity of certain antibiotics and therefore introduce errors into the AST. For example, the addition of thymidine necessary for the growth of thymidine dependant Escherichia coli, antagonises the antimicrobial action of trimethoprim and sulphonamides and leads to the reporting of false resistance results. It is recommended therefore, that tests must be performed to ascertain the degree of their effects.

Supplements that can be utilised in conjunction with Isotonic Sensitivity Test Agar\(^1\) (effects on the AST must be ascertained prior to use).

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Nutrient</th>
</tr>
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<tbody>
<tr>
<td>Symbiotic Streptococci</td>
<td>Pyridoxine Hydrochloride (1μg/ml)</td>
</tr>
<tr>
<td>Strains of Enterobacteriaceae forming dwarf colonies on routine media (e.g. E.coli, Citrobacter, Klebsiella)</td>
<td>Thymidine</td>
</tr>
<tr>
<td>Strains of Staphylococcus formning dwarf colonies</td>
<td>Thiamine (2μg/ml), Menadione (0.5 μg/ml)</td>
</tr>
<tr>
<td>Neisseria, Streptococcus Haemophilus</td>
<td>Lysed Horse Blood (5%)</td>
</tr>
</tbody>
</table>

References

6. Duncan IBR. Susceptibility of 1,500 isolates of Pseudomonas aeruginosa to gentamicin, carbenecillin, colistin and polymyxin B. Antimicrobial Agents and Chemotherapy. 1974; (Jan): 9-15

*Formulation may change to meet performance criteria

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