*Medium 18. Urea Broth Medium*

Urea Broth Medium is recommended for the identification of bacteria on the basis of urea utilization, specifically for the differentiation of *Proteus* species from *Salmonella* and *Shigella* species in accordance with Indian Pharmacopoeia, 2007.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium dihydrogen orthophosphate</td>
<td>9.100</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.100</td>
</tr>
<tr>
<td>Anhydrous disodium hydrogen phosphate</td>
<td>9.500</td>
</tr>
<tr>
<td>Urea</td>
<td>20.000</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.010</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 38.71 grams in 1000 ml purified/distilled water. Mix thoroughly to dissolve the medium completely. Sterilize by filtration. Aseptically dispense in sterile tubes as desired.

**Principle And Interpretation**

Urea Broth Medium was developed by Rustigian and Stuart (1). This medium is especially recommended by Indian Pharmacopoeia (5) for the differentiation of *Proteus* species from *Salmonella* and *Shigella* species in the enteric infection diagnosis (2), based on urea utilization (3, 4). It is also recommended for microbial limit tests. Other Gram-negative enteric bacilli are unable to utilize urea and fail to grow because of reduced availability of other nutrients.

Urea Broth Medium becomes alkaline as the utilization of urea by the organisms liberate ammonia during the incubation, indicated by pink red colour. All urea test media rely on the alkalinity formation and so they are not specific for urease testing. Yeast extract provides essential vitamins and other growth factors. Phosphates aids as good buffering agent.

The utilization of proteins may raise the pH to alkalinity due to protein hydrolysis and excess of amino acids results in false-positive reaction. This medium shows positive reaction with Genus *Proteus*, few *Providencia* and *Morganella* sp. species

**Quality Control**

**Appearance**
Light yellow to light pink homogeneous free flowing powder

**Colour and Clarity of prepared medium**
Yellow orange coloured clear solution

**Growth Promotion Test**
Growth Promotion is carried out in accordance with Indian Pharmacopoeia

**Cultural Response**
Cultural characteristics observed after an incubation at 36-38°C for 18-24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Urease</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>50-100</td>
<td>Negative reaction, no change</td>
</tr>
<tr>
<td><em>ATCC 13048</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em></td>
<td>50-100</td>
<td>Negative reaction, no change</td>
</tr>
<tr>
<td><em>ATCC 14028</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Proteus vulgaris ATCC 13315**
50-100 Positive reaction, cerise colour

**Klebsiella pneumoniae ATCC 13883**
50-100 Positive reaction, cerise colour

**Escherichia coli ATCC 8739 50-100**
Negative reaction, no change

**Klebsiella pneumoniae ATCC 10031**
50-100 Positive reaction, cerise colour

**Escherichia coli NCTC 9002 50-100**
Negative reaction, no change

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

**Reference**
5. Indian Pharmacopoeia, 2007 Govt. of India, Ministry of Health and Family Welfare, New Delhi, India.