Urea Broth Base (Diagnostic Stuarts Urea Broth Base)

Intended Use:
Urea Broth 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.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monopotassium phosphate</td>
<td>9.100</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>9.500</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.100</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.010</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.8±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 18.71 grams in 950 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 55°C. Aseptically add 50 ml of sterile 40% Urea solution (FD048). Mix well and distribute in 10 ml amounts into sterile tubes.

Principle And Interpretation
Rustigian and Stuart developed Urea Broth (1). This medium is especially recommended for the differentiation of *Proteus* species from *Salmonella* and *Shigella* species in the enteric infection diagnosis (2), based on urea utilization (3, 4). Gram-negative enteric bacilli are unable to utilize urea because of less nutrients and high buffering capacity of the medium. Urea Broth becomes alkaline as the utilization of urea by the organisms liberates 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. The utilization of proteins may raise the pH to alkalinity due to protein hydrolysis and excess of amino acids results in false-positive reaction. A medium without urea serves as negative control to rule out false positive results.

Type of specimen
Pure isolate

Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,2,8). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(3)

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. Prolonged incubation may cause alkaline reaction in the medium.
2. Also, all urea test media rely on the alkalinity formation and so they are not specific for determining the absolute rate of urease activity (4).

Please refer disclaimer Overleaf.
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
Light yellow to light pink homogeneous free flowing powder

Colour and Clarity of prepared medium
Yellowish orange coloured clear solution in tubes.

Reaction
Reaction of basal medium (1.87gm in 95ml distilled water) at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
Cultural characteristics observed on addition of sterile 40% Urea solution (FD048) after an incubation at 35-37°C for 18-24 hours.

Cultural Response

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Urease</th>
</tr>
</thead>
<tbody>
<tr>
<td># Klebsiella aerogenes ATCC 13048 (00175*)</td>
<td>50-100</td>
<td>negative reaction, no change</td>
</tr>
<tr>
<td>Escherichia coli ATCC 8739 50-100 (00012*)</td>
<td></td>
<td>negative reaction, no change</td>
</tr>
<tr>
<td>Klebsiella pneumonia ATCC 10031</td>
<td>50-100</td>
<td>positive reaction, cerise colour</td>
</tr>
<tr>
<td>Escherichia coli NCTC 9002 50-100</td>
<td></td>
<td>negative reaction, no change</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>negative reaction, no change</td>
</tr>
<tr>
<td>Klebsiella pneumonia ATCC 13883 (00097*)</td>
<td>50-100</td>
<td>positive reaction, cerise colour</td>
</tr>
<tr>
<td>Proteus vulgaris ATCC 13315</td>
<td>50-100</td>
<td>positive reaction, cerise colour</td>
</tr>
<tr>
<td>Salmonella Typhimurium ATCC 14028 (00031*)</td>
<td>50-100</td>
<td>negative reaction, no change</td>
</tr>
<tr>
<td>Proteus mirabilis ATCC 25933</td>
<td>50-100</td>
<td>Positive reaction, cerise colour</td>
</tr>
</tbody>
</table>

Key : *Corresponding WDCM numbers.

#- Formerly known as Enterobacter aerogenes

Storage and Shelf Life

Store below 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

Please refer disclaimer Overleaf.
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 (6,7).

**Reference**