Rapid Urease Test Broth

**Intended Use**
Recommended for the differentiation of organisms, especially the *Enterobacteriaceae* on the basis of urease production.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>0.100</td>
</tr>
<tr>
<td>Urea</td>
<td>20.000</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>0.091</td>
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<tr>
<td>Disodium hydrogen phosphate</td>
<td>0.095</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>

**Directions**
Suspend 20.30 grams in 1000 ml purified / distilled water. Mix well and sterilize by filtration. DO NOT AUTOCLAVE OR HEAT THE MEDIUM. Dispense in sterile tubes or flasks as desired.

**Principle And Interpretation**
In Rapid Urease test Broth the urease reaction given by *H. pylori*, occurs more quickly than that seen by other organisms which may split urea. As a result, it is an effective presumptive test for the presence of *H. pylori*. It is also used for the rapid detection of urease activity in bacteria such as *Proteus* spp., or in yeast, (such as *Cryptococcus neoformans*).

*Helicobacter pylori* is a gram negative, curved, microaerophilic and motile organism with multiple polar flagella.

*Helicobacter pylori* is a spiral urease producing organism that lies in the interface between gastric epithelial cell surface and the overlying mucus gel (5). It resides in the stomach of man and other primates, lining up the gastric mucus secreting cells. Rapid urease test is one of the invasive tests. This method has been used to help simplify the diagnosis of *H. pylori*, especially those specimens originating from duodenal and gastric ulcers, and chronic antral gastritis (type B). This medium is develop as per McFaddin (4). Urease activity can be described as the splitting of urea via hydrolysis by a urease enzyme. The end products from this reaction yield ammonium carbonate and ammonia, which are alkaline in nature. The consequent rise in the pH of the medium is detected by phenol red indicator. The test is non-toxic, and the pH change that occurs from accumulation of alkaline end products is detected by a pH indicator in the media (3). *Helicobacter pylori* is an organism that may be easily identified by this test because of its very high endogenous urease activity.

Yeast extract which provides nitrogen and vitamin required for growth. Phosphates serve to buffer the medium.

**Type of specimen**
Isolated Microorganism

**Specimen Collection and Handling**
For isolated Microorganism, follow appropriate techniques for sample collection, processing as per guidelines and local standards(4). After use, contaminated materials must be sterilized by autoclaving before discarding.

**Warning and Precautions**
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 specimens. Safety guidelines may be referred in individual safety data sheets.

**Limitations**
1. The Urease test is pH dependant, accumulation of alkaline end products may interfere with the positive results
2. Further biochemical and serological testing is required for complete identification.
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 100ml distilled water) at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 4-18 hours.

Organism Inoculum (CFU) Urease

Klebsiella aerogenes ATCC 13048 (00175*) 50-100 negative reaction, no change

Escherichia coli ATCC 25922 (00013*) 50-100 negative reaction, no change

Klebsiella pneumoniae ATCC 13883 (00097*) 50-100 weak positive reaction

Proteus vulgaris ATCC 13315 50-100 positive reaction, cerise colour

Salmonella Typhimurium ATCC 14028 (00031*) 50-100 negative reaction, no change

Helicobacter pylori ATCC 43504 50-100 positive reaction, cerise colour

Klebsiella pneumoniae ATCC 10031 50-100 weak positive reaction

Key : (*) Corresponding WDCM numbers. (#) Formerly known as Enterobacter aerogenes

Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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. 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 (1,2).

Reference