**PYR Broth**

**Intended Use**

Recommended for isolation and identification of *Streptococcus pyogenes*.

**Composition**

**Ingredients**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM infusion from #</td>
<td>500.000</td>
</tr>
<tr>
<td>Peptone</td>
<td>20.000</td>
</tr>
<tr>
<td>Dextrose (Glucose)</td>
<td>2.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2.000</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>0.400</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>2.500</td>
</tr>
<tr>
<td>Chromogenic mixture</td>
<td>0.100</td>
</tr>
<tr>
<td><strong>Final pH (at 25°C)</strong></td>
<td><strong>7.8±0.2</strong></td>
</tr>
</tbody>
</table>

# Equivalent to Beef heart infusion from

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

**Directions**

Suspend 37 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Mix well and dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

**Principle And Interpretation**

PYR hydrolysis is a presumptive test for both group A and group D enterococcal streptococci (1). The PYR test determines the activity of enzyme L-pyrrolidonyl arylamidase (PYR) produced by *Streptococcus pyogenes* but not by other β-haemolytic streptococci (5). Free β-naphthylamide is then detected by addition of the diazo dye complex, N,N-dimethylaminocinnamaldehyde. Development of a red colour is indicative of PYR hydrolysis (4). PYR test is a highly sensitive test, which replaces bacitracin and salt tolerance (growth in 6.5% NaCl) tests (1). PYR Broth is recommended for detection and presumptive identification of *S. pyogenes* based on PYR hydrolysis (6).

Todd Hewitt Broth Base (M313) acts as the basal medium to which substrate for PYR enzyme is added (4). HM infusion from and Peptone provide nitrogenous nutrients, long chain amino acids and vitamins. Dextrose (Glucose) is the carbohydrate serving as an energy source. Disodium phosphate serves as buffering agent and sodium chloride maintains osmotic balance. Chromogenic mixture provides substrate for PYR enzyme. After an incubation at 35-37°C for 18-24 hours, add 1 drop of PYR reagent (R043) directly to suspected surface growth on plate. Observe for colour change after 2 minutes. The chromogenic mixture is hydrolysed by *S. pyogenes* to L-pyrrolidone and β-naphthylamine. The PYR reagent reacts with β-naphthylamine to form a red coloured Schiff's Base indicating a positive reaction.

**Type of specimen**

Pure isolates.

**Specimen Collection and Handling**

For pure isolate follow appropriate techniques for handling specimens as per established guidelines (5). 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. Few *Staphylococcus, Aerococcus, Lactococcus*, most *Corynebacterium (Arcanobacterium) haemolyticum*, and *Enterobacteriaceae* are also PYR-positive which needs further confirmation.
2. Further biochemical and serological tests must be carried out 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**
Cream to yellow homogeneous free flowing powder

**Colour and Clarity of prepared medium**
Light yellow coloured clear solution

**Reaction**
Reaction of 3.7% w/v aqueous solution at 25°C. pH : 7.8±0.2

**pH**
7.60-8.00

**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>PYR (on addition of PYR reagent, R044)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pyogenes</em> ATCC 19615</td>
<td>50-100</td>
<td>luxuriant</td>
<td>positive, red colouration</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 50-100 (00087*)</td>
<td>luxuriant</td>
<td></td>
<td>positive, red colouration</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>negative</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em> ATCC 12386</td>
<td>50-100</td>
<td>luxuriant</td>
<td>negative</td>
</tr>
</tbody>
</table>

Key : (*) Corresponding WDCM numbers.

**Storage and Shelf Life**
Store dehydrated powder 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 (2,3).

**Reference**


Please refer disclaimer Overleaf.