RS Medium Base (Rimler-Shotts Medium Base)  

**Intended use**

Rimler-Shotts (RS) Medium Base is used for selective isolation, cultivation and presumptive identification of *Aeromonas hydrophila* from clinical and non-clinical samples.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Maltose</td>
<td>3.500</td>
</tr>
<tr>
<td>L-Cysteine hydrochloride</td>
<td>0.300</td>
</tr>
<tr>
<td>L-Lysine hydrochloride</td>
<td>5.000</td>
</tr>
<tr>
<td>L-Ornithine hydrochloride</td>
<td>6.500</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>6.800</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>0.800</td>
</tr>
<tr>
<td>Sodium deoxycholate</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.030</td>
</tr>
<tr>
<td>Agar</td>
<td>13.500</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 45.43 grams in 990 ml purified / distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and aseptically add rehydrated content of 1 vial of Novobiocin Supplement (FD096). Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

RS Medium was formulated by Rimler and Shotts (6) based on the principle of Xylose-Lysine (XL) Agars (7,8). It is used for selective isolation and presumptive identification of *Aeromonas hydrophila* and other gram-negative bacteria based on their ability to decarboxylate lysine and ornithine, maltose fermentation and H2S production (4). Yeast extract acts as a source of nutrients. Sodium thiosulphate, L-cysteine hydrochloride and ferric ammonium citrate are the indicators of H2S production. The medium contains maltose, which is mostly fermented by all *Aeromonas*. Maltose fermentation is indicated by bromothymol blue. Sodium deoxycholate and novobiocin inhibit gram-positive bacteria and *Vibrio* species. *Citrobacter freundii* usually produce H2S but occasionally negative strains exist. The medium contains L-cysteine and L-ornithine, which are often decarboxylated by enteric bacteria to give alkaline products. Lysine positive and ornithine positive strains of *Aeromonas* may not have the typical strong yellow colour because of alkaline products produced during decarboxylation of the amino acids. Results are interpreted within 24 hours since after 26 hours slow reversion of yellow colour to a basic (green) colour occurs. Medium should be incubated at 35°C, which will eliminate possible growth of *Aeromonas salmonicida*, which may grow at reduced temperatures giving false-positive reaction. Test the yellow colonies with or without black centers (of H2S) for oxidase to rule out *Citrobacter* species. *Proteus mirabilis* is inhibited on this medium.

**Type of specimen**

Clinical samples - stool, Food samples ; Water samples.

**Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3 ). For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(1) After use, contaminated materials must be sterilized by autoclaving before discarding.
Warning and Precautions
In Vitro diagnostic use. Read the label before opening the container. The media should be handled by trained personnel only. Wear protective gloves protective clothing eye protection face protection. Allow 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. Pure isolate must be used
2. Results are interpreted within 24 hours since after 26 hours slow reversion of yellow colour to a basic (green) colour occurs.

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 green homogeneous free flowing powder

Gelling
Firm, comparable with 1.35% Agar gel.

Colour and Clarity of prepared medium
Dark green coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.54% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed with added Novobiocin Supplement(FD096) after an incubation at 35-37°C for 24 hours

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Maltose fermentation</th>
<th>Lysine/Ornithine decarboxylation</th>
<th>H2S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeromonas hydrophila ATCC 7966 (00063*)</td>
<td>50-100</td>
<td>good</td>
<td>positive reaction, yellow reaction</td>
<td>negative reaction</td>
<td>negative reaction</td>
</tr>
<tr>
<td>Citrobacter freundii ATCC 8090</td>
<td>50-100</td>
<td>good</td>
<td>negative reaction</td>
<td>variable reaction</td>
<td>positive, black centered colonies</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>good</td>
<td>negative reaction</td>
<td>variable reaction</td>
<td>negative reaction</td>
</tr>
<tr>
<td>Proteus vulgaris ATCC 13315</td>
<td>50-100</td>
<td>good</td>
<td>positive reaction, yellow reaction</td>
<td>negative reaction</td>
<td>positive, black centered colonies</td>
</tr>
<tr>
<td>Salmonella Typhi ATCC 6539</td>
<td>50-100</td>
<td>good</td>
<td>positive reaction, yellow reaction</td>
<td>negative reaction</td>
<td>negative reaction</td>
</tr>
</tbody>
</table>

Key : * Corresponding WDCM numbers

Please refer disclaimer Overleaf.
Storage and Shelf Life
Store between 10-30°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
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).

References

Revision : 02 / 2019
Disclaimer:

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.