Ampicillin Selective Supplement

PRODUCT INFORMATION
A043-5g - Ampicillin Anhydrous, Powder, 5g
A043-25g - Ampicillin Anhydrous, Powder, 25g
A042-5g - Ampicillin Sodium, Powder, 5g
A042-25g - Ampicillin Sodium, Powder, 25g
A042-100g - Ampicillin Sodium, Powder, 100g
A009-5g - Ampicillin Trihydrate, Powder, 5g
A009-25g - Ampicillin Trihydrate, Powder, 25g
A009-100g - Ampicillin Trihydrate, Powder, 100g

DESCRIPTION
Aeromonas medium base (Ryan) with ampicillin selective supplement is a selective diagnostic medium for the isolation of Aeromonas hydrophila from clinical and environmental specimens when used with Ampicillin Selective Supplement.

BACKGROUND
Ampicillin is a beta-lactam antibiotic that has been used extensively to treat bacterial infections since 1961. Until the introduction of ampicillin by the British company Beecham, penicillin therapies had only been effective against Gram-positive organisms such as staphylococci and streptococci. Ampicillin (originally branded as 'Penbritin') also demonstrated activity against Gram-negative organisms such as H. influenzae, coliforms and Proteus spp. Ampicillin was the first of a number of so-called broad spectrum penicillins subsequently introduced by Beecham. Ampicillin is part of the aminopenicillin family and is roughly equivalent to its successor, amoxicillin in terms of spectrum and level of activity.

Mechanism of action
Belonging to the penicillin group of beta-lactam antibiotics, ampicillin is able to penetrate Gram-positive and some Gram-negative bacteria. It differs from penicillin only by the presence of an amino group. That amino group helps the drug penetrate the outer membrane of gram-negative bacteria.

Ampicillin acts as a competitive inhibitor of the enzyme transpeptidase, which is needed by bacteria to make their cell walls. It inhibits the third and final stage of bacterial cell wall synthesis in binary fission, which ultimately leads to cell lysis. Ampicillin has received FDA approval for its mechanism of action.

APPLICATION IN AEROMONAS MEDIUM BASE (RYAN)
Aeromonas medium base (Ryan) is a selective diagnostic medium for the isolation of Aeromonas hydrophila from clinical and environmental specimens when used with ampicillin.

Ryan modified the formulation of XLD medium so that it would support the growth of Aeromonas spp and Plesiomonas spp as well as the usual Enterobacteriaceae. It could therefore be used as a universal medium in the investigation of enteric disease. However, to improve its performance in the isolation of aeromonads, the addition of ampicillin at 5 mg/l is recommended. The effectiveness of ampicillin as a selective agent for Aeromonas spp has been reported by several workers. The value of Aeromonas medium base (Ryan) is that the recommended level of ampicillin is well below that which can cause inhibition of some strains of aeromonad.

The utility of Aeromonas medium (Ryan) and its superiority over some other formulae for detection of Aeromonas spp in tap water, bottled water and foods including meat, poultry, fish and seafoods has been reported. Aeromonas medium (Ryan) is specified by the MAFF/DHS Steering Group on the Microbiological Safety of Food for detection and enumeration of Aeromonas hydrophila in clinical specimens. Aeromonas spp occur widely in soil and water, where they cause diseases in fish and amphibians. They also occur in untreated and chlorinated drinking water, raw foods and raw milk. It is considered that the major cause of gastrointestinal infections by Aeromonas spp is from ingesting infected water.

The role of these organisms in gastrointestinal disease is still subject to debate but a rapidly expanding body of literature suggests that Aeromonas spp can cause a wide spectrum of enteric symptoms in adults as well.
as children. It would therefore be a useful diagnostic aid to include this selective medium when investigating diarrhoeal disease.

**Content concentrations**

<table>
<thead>
<tr>
<th>Typical Formula*</th>
<th>mg/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteose peptone</td>
<td>5</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3</td>
</tr>
<tr>
<td>L. Lysine monohydrochloride</td>
<td>3.5</td>
</tr>
<tr>
<td>L. Arginine monohydrochloride</td>
<td>2</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>3</td>
</tr>
<tr>
<td>Inositol</td>
<td>2.5</td>
</tr>
<tr>
<td>Lactose</td>
<td>1.5</td>
</tr>
<tr>
<td>Xylose</td>
<td>3.75</td>
</tr>
<tr>
<td>Bile Salts No.3</td>
<td>3</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>10.67</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>0.8</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.04</td>
</tr>
<tr>
<td>Thymol blue</td>
<td>0.04</td>
</tr>
<tr>
<td>Agar</td>
<td>12.5</td>
</tr>
</tbody>
</table>

**Ampicillin Selective Supplement**

| Ampicillin        | 5 |

* Adjusted as required to meet performance standards

| Table 1 typical formula for Aeromonas medium base (Ryan) and Ampicillin selective supplement |

**METHOD**

**Preparation**

Suspend appropriate amount of Aeromonas medium base (Ryan) in distilled water. Bring gently to the boil. Do not autoclave. Cool to 50 °C and aseptically add appropriate amount of ampicillin reconstituted as directed. Mix well and pour plates.

**Protocol**

1. Prepare the medium according to preparation and pour into sterile dishes. The prepared medium may be stored at 2-8 °C up to 5 days.

2. Inoculate the plates with a suspension of food, faeces etc., diluted to form single colonies on the inoculated plate.

3. Incubate the plates aerobically at 30-35 °C for 24 hours. If further incubation is required hold at room temperature (22-25 °C).

4. Examine the plates for the presence of dark green, opaque colonies with darker centres. Confirm the identity with biochemical tests.

The typical colonial appearance of *Aeromonas* isolates on this medium is as follows:

- **Aeromonas** species: dark green, opaque with darker centre, diameter 0.5-1.5 mm.
- **Pseudomonas** species: blue/grey translucent, diameter from pinpoint to 0.25 mm.

**Quality control**

**Positive control:**

*Aeromonas hydrophila* ATCC® 7966: Good growth; opaque green colonies with dark centres

**Negative control:**

*Escherichia coli* ATCC® 11775: No growth

**REFERENCES**