**Introduction**

Chronic respiratory disease is the primary contributing factor in the premature death of patients with Cystic Fibrosis. Recently *Burkholderia cepacia* (previously known as *Pseudomonas cepacia*) has emerged as a potentially important respiratory pathogen appearing with increasing frequency.

*B.cepacia* is widely distributed throughout the environment and nutritionally is extremely versatile. The organism has been isolated from natural and tap water, soil and, increasingly, from hospital environments. It is not a common human pathogen but poses a major threat to patients with Cystic Fibrosis, as once *B.cepacia* colonises a patient it is very difficult to eradicate, partly due to its resistance to a variety of antimicrobial agents.

Nearly all strains appear resistant to Ticarcillin, Carbenicillin and aminoglycosides. Many isolates also show resistance to Chloramphenicol (92%), Trimethoprim/Sulphamethoxazole (85%) and Ceftazidime which have been indicated as having good *in-vitro* activity against *B.cepacia*.

Gilligan *et al.* produced a medium to selectively isolate *B.cepacia* using Polymyxin B (300,000 units/litre) together with Ticarcillin (100mg/litre) as selective agents. This medium selectively supported growth of *B.cepacia* from clinical samples whilst inhibiting many of the organisms frequently found in respiratory secretions of patients with Cystic Fibrosis.

**Description**

MAST has available supplements for the selective culture of *B.cepacia*. *Burkholderia cepacia Selectatab™* is designed for direct addition to 100ml of MAST Burkholderia cepacia Medium (DM253). *Burkholderia cepacia Selectavial™* is designed for addition to 500ml of medium after reconstitution with an approved diluent.

**MS22, SV22** For the selective isolation of *Burkholderia (Pseudomonas) cepacia*.

**MS22**

<table>
<thead>
<tr>
<th>Content</th>
<th>Concentration in 100ml medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ticarcillin</td>
<td>10mg 100mg/litre</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>30,000 units 300,000 units/litre</td>
</tr>
</tbody>
</table>

**SV22**

<table>
<thead>
<tr>
<th>Content</th>
<th>Concentration in 500ml medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ticarcillin</td>
<td>50mg 100mg/litre</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>150,000 units 300,000 units/litre</td>
</tr>
</tbody>
</table>

**Directions**

1. Label Petri dishes using the self-adhesive labels provided.
2. Sterilise the medium, MAST Burkholderia cepacia Medium (DM253), cool to 50-55°C and hold in a water bath at this temperature.
3. Using sterile forceps add one Selectatab™ to each 100ml of medium and label the bottle. Allow to stand for several minutes in the water bath until the Selectatab™ has broken up.
4. After the Selectatab™ has broken up swirl 3-4 times and invert to complete dispersal.
   An alternative method is to first dissolve the Selectatab™ in 3-5ml of sterile water and add this to the appropriate volume of medium.
5. Mix well, pour culture plates of normal thickness (15-20ml per plate) and allow to set.
6. Prepared culture plates may be used immediately or stored in plastic bags at 2-8°C for up to one week before use.
Selectavial™

1. Sterilise the appropriate volume of MAST Burkholderia cepacia Medium (DM253), cool to 50-55°C and hold in a water bath at this temperature.

2. Reconstitute the contents of one vial using 5ml of sterile water. The best method is to aseptically add the diluent using a sterile needle and syringe. Draw the diluent into the syringe and after removing the plastic cap of the vial, inject through the rubber stopper of the vial. The lyophilised supplement will rapidly dissolve and may be withdrawn into the syringe.

3. Add the antibiotic supplement to 1 litre of medium and discard the needle into an approved container.

4. Mix gently but thoroughly to evenly distribute the selective agents. Pour culture plates of normal thickness (15-20ml) and allow to set.

5. Prepared culture plates may be used immediately or stored in plastic bags at 2-8ºC for up to one week before use.

In Use

Liquefy sputum by the addition of a suitable liquefying agent (Mast Sputagest™ Selectavial SV40). If required (see below) dilute 0.1ml of the resulting sample in 9.9ml of physiological saline or Ringer’s solution. Further dilute this sample by transferring 0.1ml to 9.9ml of diluent to provide final dilutions of 1 in 200 and 1 in 2000 respectively. (The sample is initially diluted 1 in 2 during liquefaction).

For quantitative investigations inoculate additional plates with the prepared dilutions. Plates should be incubated and examined after 24 and 48 hours at 37°C and then for a further 5 days at room temperature before being discarded.

Colonies of B.cepacia will grow up to 1-2mm in diameter, the medium often turning pink to purple especially in areas of heavy growth. Occasionally growth by some strains of Candida spp., Stenotrophomonas maltophilia, Comomonas acidovorans, multi-resistant Pseudomonas aeruginosa and Ps.putida may occur on the medium but generally organisms other than B.cepacia will be strongly inhibited.

References