MS29, SV29. For use in the detection of methicillin resistant *Staphylococcus aureus* (MRSA).

**Introduction**

Infections caused by *Staphylococcus aureus* strains resistant to methicillin and other beta-lactams have remained a significant nosocomial problem since their emergence in the early 1960's. It is of increasing importance that such strains be quickly and accurately recognised, both for the appropriate selection of antimicrobial agents for therapy and for hospital infection control.

Laboratory recognition of methicillin resistant strains of *Staph. aureus* (MRSA) may however prove to be a problem. In a culture containing both methicillin resistant and susceptible organisms, unless special test conditions are used, the resistant portion of the cell population will grow more slowly and therefore be overgrown by the faster growing, susceptible, sub-population preventing identification of the resistant strains.

The use of Mueller Hinton Broth with 5% added salt was investigated by Barry *et al.* in an attempt to improve the isolation rate of MRSA. Addition of salt appeared to enable earlier detection of resistant strains as well as increase the stability of the antibiotic agents used. More recent work by Lally *et al.* has shown that the use of oxacillin at a final concentration of 4mg/l together with Mannitol Salt Agar provides a reliable screening medium for the simultaneous detection and identification of MRSA. A similar method, utilising a lower concentration of oxacillin (2mg/l), has been recommended by the Public Health Laboratory Service (PHLS) in the UK for the selective isolation of methicillin resistant *Staphylococcus aureus*.

**Description**

MAST has available supplements for the detection of MRSA which can be used to prepare media in accordance with either of the above methods simply by altering the volume of the basal medium used. Each MAST MRSA Selectatab™ is designed for direct addition to either 100ml or 200ml of MAST Mannitol Salt Agar (DM160), while the MRSA Selectavial™ is designed for addition to 1 or 2 litres of medium after reconstitution with an approved diluent, depending on the method chosen. The table below illustrates the alternative usage of the products.

<table>
<thead>
<tr>
<th></th>
<th>MAST MRSA Selectatab™</th>
<th>Concentration in 100ml medium</th>
<th>Concentration in 200ml medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS29</td>
<td>Oxacillin</td>
<td>0.4mg</td>
<td>4mg/litre</td>
</tr>
<tr>
<td>SV29</td>
<td>Oxacillin</td>
<td>4mg</td>
<td>2mg/litre</td>
</tr>
</tbody>
</table>

**Directions**

**Selectatab™**

1. Label Petri dishes using the self-adhesive labels provided.
2. Sterilise the medium, MAST Mannitol Salt Agar (DM160), cool to 50-55°C and hold in a water bath at this temperature.
3. Using sterile forceps add one Selectatab™ to the appropriate volume of medium and label the bottle. Allow to stand for several minutes in a 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) 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 Mannitol Salt Agar (DM160), 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 the 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

Inoculate the organism onto screening plates containing Mannitol Salt Agar and oxacillin (MSA + OX) and growth control plates of Mannitol Salt Agar (MSA) only.

The medium is also suitable for direct culture of clinical specimens and for subculture from suitable enrichment broths such as nutrient broth containing 7.5% NaCl.

Incubate plates for up to 48 hrs at 37ºC and examine carefully. A positive result is indicated by yellow growth on both the MSA + OX and MSA plates. A negative result is indicated by no growth on the MSA + OX plates and yellow growth on the MSA control plate. Organisms failing to grow on the MSA control plate should be rechecked for identity or viability.

Multipoint inoculation is also suitable and economical.

References