Haemophilus Selectatab™
Haemophilus Selectavial™

MS27, MS27A, SV27 For the selective isolation and culture of *Haemophilus* spp.

Introduction

The genus *Haemophilus* contains a number of species which are important human and animal pathogens. However, the lack of an effective selective medium in the past may have hindered the identification and assessment of the role of some of these species such as *Haemophilus influenzae* and *Haemophilus ducreyi*.

Haemophili are non-motile, Gram negative organisms which are strictly parasitic and which, when being cultured *in-vitro*, require the provision of growth factors present in the blood: X factor (Haemin) and / or V factor (NAD). Difficulties are often encountered in culturing *Haemophilus* spp. due to overgrowth by more strongly growing commensal organisms particularly when specimens from the respiratory tract are being examined.

Various workers\(^1,2\) identified bacitracin as a selective agent able to allow the growth of *Haemophilus* spp. while suppressing the growth of Gram positive bacteria and *Neisseria* spp although the concentrations of antibiotic chosen varied considerably from 100 to 375mg/l. Ederer and Schurr\(^3\) examined the effect of these widely different bacitracin concentrations on the isolation rate and growth of Haemophilus. They reported consistently more profuse growth of the organisms at a concentration of 100mg/l and a poorer isolation rate at 300mg/l, the highest concentration tested.

More recent work by Roberts , Higgs and Cole\(^4\), in which bacitracin was used at a concentration of 100mg/litre in the development of a selective medium for the isolation and differentiation of *Haemophilus* spp., confirmed the value of this antibiotic content for the recovery of haemophilus from the respiratory tract.

Media for the isolation of Haemophilus is commonly prepared by heating blood-containing media to produce "chocolate agar". The process inactivates NADase and releases extra V factor into the medium but produces plates dark brown in colour. For certain samples an enriched blood medium, with the inclusion of pure X and V factors (SV62 Haemophilus Growth Supplement), is often preferred. This facilitates identification of other pathogens that may be present in a specimen, for instance from oropharyngeal or conjunctival swabs, and allows visualisation of haemolytic activity in *H. haemolyticus* and *H.parahaemolyticus*. Both these bases have been used with the addition of bacitracin\(^2,3,5\) to prepare selective media for the culture of *Haemophilus* and can be used in conjunction with Mast Haemophilus Selectavial or Selectatab.

Description

MAST has available a range of supplements for the selective culture of *Haemophilus* spp. They are accurately assayed quantities of bacitracin in lyophilised tablet form (Selectatab\(^\text{™}\)) for direct addition to 100ml (MS27) or 500ml (MS27A) of medium and lyophilised in vials (Selectavial\(^\text{™}\)) SV27 for direct addition to 1 litre of medium following reconstitution with an approved diluent.

<table>
<thead>
<tr>
<th>MS27</th>
<th>Content</th>
<th>Concentration in 100ml medium</th>
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<tbody>
<tr>
<td><strong>Haemophilus Selectatab™</strong></td>
<td>Bacitracin 10mg</td>
<td>100mg/litre</td>
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<tr>
<th>MS27A</th>
<th>Content</th>
<th>Concentration in 500ml medium</th>
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<tbody>
<tr>
<td><strong>Haemophilus Selectatab™</strong></td>
<td>Bacitracin 50mg</td>
<td>100mg/litre</td>
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<thead>
<tr>
<th>SV27</th>
<th>Content</th>
<th>Concentration in 1000ml medium</th>
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</thead>
<tbody>
<tr>
<td><strong>Haemophilus Selectatab™</strong></td>
<td>Bacitracin 100mg</td>
<td>100mg/litre</td>
</tr>
</tbody>
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Directions

Selectatab™

1. Label Petri dishes using the self-adhesive labels provided.

2. Sterilise the appropriate volume of MAST Columbia Agar (DM115) and cool to 50-55°C. Add 5-7% sterile defibrinated horse blood and mix thoroughly.
3. To prepare an enriched blood medium add Haemophilus Growth Supplement (SV63) - see appropriate instructions - or, alternatively, to prepare chocolate agar heat the medium to 80°C mixing occasionally until the medium becomes a chocolate brown colour and allow the medium to cool to 55°C.

4. Using sterile forceps add one Haemophilus Selectatab™ to the appropriate volume of medium. Allow to stand for several minutes in the water bath until the Selectatab™ has broken up.

5. 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.

6. Mix well, pour culture plates of normal thickness (15-20ml per plate) and allow to set.

7. 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 Columbia Agar (DM115), cool to 50-55°C. Add 5-7% sterile defibrinated horse blood and mix thoroughly.

2. To prepare an enriched blood medium add Haemophilus Growth Supplement (SV63) - see appropriate instructions - or, alternatively, to prepare chocolate agar heat the medium to 80°C mixing occasionally until the medium becomes a chocolate brown colour and allow the medium to cool to 55°C.

3. 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.

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

DO NOT TRY TO RE-SHEATH AN EXPOSED NEEDLE

Unused reconstituted supplement should be discarded and not frozen.

5. Mix gently but thoroughly to evenly distribute the selective agents.

6. Pour culture plates of normal thickness (15-20ml) and allow to set.

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

In Use

Inoculate the specimen directly onto the selective plates. Incubate at 37°C in an atmosphere containing 10% CO2 for 18-24 hours. Suspect colonies of Haemophilus spp. should be further tested for X and V factor requirement using Mast X and V Factor discs or Mast XV Mirror Ring (MID/XV).

References