Haemophilus Growth Supplement

SV62 For the growth enhancement of *Haemophilus* spp. on blood agar media.

**Introduction**

Members of the genus *Haemophilus* are obligate parasites usually inhabiting, as normal flora, the upper respiratory tract of humans and many animal species. The major pathogen within the genus is *Haemophilus influenzae*, exclusively a human parasite, which is associated with a wide spectrum of disease including meningitis and chronic respiratory infections.

*Haemophilus* are typically unable to grow on culture media in the absence of blood or blood products due to an *in vitro* requirement for certain growth factors. These factors were originally referred to as X and V factors and later identified as haemin and nicotinamide adenine dinucleotide (NAD) respectively. Different species require either or both of these factors and the pattern of X and V dependency, usually determined by a simple paper disc method, is extensively used for identification.

On blood agar, although X factor is directly available to the organism, growth is restricted by the limited availability of V factor which is only released by breaking up the blood cells. Colonies are consequently only small in size. The source of the blood used is also an important consideration with sheep blood, containing high levels of V factor inactivating enzyme tending to produce the poorest growth. Improved growth can easily be obtained by heating blood media to produce “chocolate agar”, which counters the effects of these enzymes releasing extra V factor into the medium and produces plates dark brown in colour. A heated blood medium is therefore often preferred to facilitate identification of other pathogens that may be present in a specimen, for instance from conjunctival swabs, and visualisation of haemolytic activity in *H. haemolyticus* and *H. parahaemolyticus*. To overcome the normal inhibition of V factor activity by unheated blood an excess of NAD has to be added. Fildes prepared a peptic digest of sheep blood which liberated the necessary V factor and also converted haemoglobin to haematin which is a much better source of the X factor required by *H. influenzae*. The use of complex growth promoting additives can, however, result in variations in medium performance from batch to batch and in order to improve the reliability of antimicrobial susceptibility testing of *Haemophilus* a simple to prepare medium of consistent performance supplemented directly with crystalline β-NAD and haemin was developed. A number of centres have recently adopted this approach to produce a horse blood Columbia Agar medium, supplemented with defined growth factors, able to satisfy the exacting nutritional requirements of *Haemophilus* spp. and suitable for the isolation of these organisms directly from clinical samples.

To enable greater recovery of *Haemophilus* spp. plates can be made selective by the inclusion of bacitracin at a final concentration of 100mg/l which inhibits much of the normal flora of the upper respiratory tract.

**Description**

MAST Haemophilus Growth Supplement contains accurately assayed quantities of NAD, Haemin and Menadione and is designed for addition to 1 litre of medium after reconstitution with an approved diluent.

<table>
<thead>
<tr>
<th>SV62 Haemophilus Growth Supplement</th>
<th>Content</th>
<th>Conc. in 1 litre medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAD</td>
<td>12.5mg</td>
<td>12.5mg/l</td>
</tr>
<tr>
<td>Haemin</td>
<td>1.25mg</td>
<td>1.25mg/l</td>
</tr>
<tr>
<td>Menadione</td>
<td>1.0mg</td>
<td>1.0mg/l</td>
</tr>
</tbody>
</table>

**Directions for Use**

1. Sterilise 1 litre of MAST Columbia Agar Base (DM115) cool the medium to 50-55°C and hold in a water bath at this temperature.

2. Reconstitute 1 vial of Haemophilus Growth Supplement with 5ml of sterile deionised water. The best method is to aseptically add 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. The lyophilised supplement will rapidly dissolve and may be drawn into the syringe.

3. Add the contents of the vial directly to 1 litre of prepared MAST Columbia Agar and discard the needle into an appropriate container. DO
NOT TRY TO SHEATH AN EXPOSED NEEDLE. Unused reconstituted supplement should be discarded and not frozen.

4. Mix gently but thoroughly to evenly distribute the components.

5. Aseptically add 5-7% v/v horse blood. Mix well.

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

For the preparation of equivalent Haemophilus Selective Agar plates also add bacitracin containing Haemophilus Selectatab™ or Selectavial™ (MS27/SV27) as described in the appropriate data sheet.

In Use

Inoculate the specimen directly onto prepared culture plates. Incubate at 37°C in an atmosphere enriched with 10% CO₂ for 18-24 hours. Suspect colonies of Haemophilus spp. should be further tested to determine X and V factor requirements using Mast X and V factor discs or cartridges (D43/D44/D45) or Mast XV Mirror Ring (MID/XV).

References