SV40 SPUTAGEST SELECTAVIAL™ is a liquefying agent for sputum to permit easier isolation of organisms responsible for chronic lung disease.

Introduction

Sputum generally consists of inflammatory exudate from the lower respiratory tract mixed with saliva. Because of its viscous nature there can be great difficulty in growing organisms directly from sputum, which is crucial to the diagnosis and management of chronic lung diseases. Bacteria are also often unevenly distributed in the sputum of patients from chronic lung bronchitis and single cultures may fail to reveal all the bacterial species present.¹

Various methods have been attempted to thin sputum, for example, homogenisation,² use of iodides and sodium salts³ but with limited success. However the use of reagents containing sulphhydryl groups have been shown to be effective as sputum liquefying agents.³ N-acetyl cysteine was usedoriginally⁴ but was later replaced with dithiothreitol (DTT). A 0.1M solution of DTT was found to achieve a significantly greater decrease in viscosity than 1.2M N-acetyl cysteine for use prior to sputum culture.³ DTT was also found to yield a higher number of organisms and size of colonies after culture than N-acetyl cysteine.⁴ DTT acts on sputum by reducing the mucoprotein cross-linking disulphide bonds.⁵

DTT is now used routinely as an agent for digesting sputum prior to processing for smears and culture, as it does not affect the morphology, growth or fluorescent antibody staining of pathogens in sputum.⁶ MAST SPUTAGEST SELECTAVIAL™ is lyophilised DTT conveniently presented in packs of 10 vials, each vial after reconstitution and dilution being sufficient to produce 100ml of liquefying agent.

Formulation

Each vial contains the following components:-

- Dithiothreitol (DTT) 0.1g
- Sodium chloride 0.78g
- Potassium chloride 0.02g
- Disodium hydrogen phosphate 0.112g
- Potassium dihydrogen phosphate 0.02g

Each vial sufficient to make 100ml of sputum digestant at a pH of 7.4 ± 0.2.

Directions

To reconstitute a vial of MAST SPUTAGEST™ aseptically add 5ml of sterile distilled or deionised water and mix gently to dissolve the contents completely. Aseptically add the vial contents to 95ml of sterile distilled or deionised water and the product is ready for use.

In Use

a) General Use

1. Sputum samples may be washed initially with saline. This can be done as follows:- add approximately 5 times the volume of 0.85% sterile saline, agitate the sample to remove any adherent saliva from the sputum, and drain off the saline with a sterile Pasteur pipette.

2. To the sputum sample add an equal volume of MAST SPUTAGEST™ shake the mixture well or vortex briefly and incubate in a 37°C water bath with periodic shaking until liquefaction is complete. Inoculate onto a suitable culture medium to grow any organisms present.

   Note prolonged standing will not inhibit floral multiplication.

b) For isolation of Predominant Organisms:

1. Add an equal volume of MAST SPUTAGEST™ to the sputum sample and leave to liquefy as above (a.2).

2. Centrifuge the mixture for 5 minutes at 1500 rpm to sediment bacterial cells.

3. Discard the supernatant and resuspend the sediment in a small amount of MAST SPUTAGEST™. The volume of diluent used will depend on the volume of sediment and the final concentration desired. A dilution of 1:100 with an inoculum of 0.01ml is recommended for colony counting. For an accurate count serial dilutions should be made.
c) For Acid Fast Bacilli:

1. Liquefy the sputum sample and centrifuge as above (b.1 and 2).

2. Decontaminate the specimen by a standard method. For example: resuspend the sediment in 5-10ml of 1% NaOH solution. Mix the specimen thoroughly and incubate.

3. Centrifuge the specimen for 15 minutes at 3000 rpm and discard the supernatant.

4. Wash the sediment twice by resuspension and centrifugation with 10ml each time of diluted MAST SPUTAGEST™.

5. Finally resuspend the sediment in 0.5ml of diluted MAST SPUTAGEST™.

6. Culture for Acid Fast Bacteria on appropriate media e.g. MAST Egg Media.

MAST manufactures a wide range of prepared Egg Media for the isolation of Mycobacteria.

<table>
<thead>
<tr>
<th>Media Type</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Löwenstein Jensen Medium</td>
<td>EM100</td>
</tr>
<tr>
<td>Löwenstein Jensen Medium with pyruvate</td>
<td>EM102</td>
</tr>
<tr>
<td>Acid Egg Medium</td>
<td>EM200</td>
</tr>
<tr>
<td>Acid Egg Medium with pyruvate</td>
<td>EM202</td>
</tr>
<tr>
<td>Dorset's Egg Medium</td>
<td>EM300</td>
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</tbody>
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Storage

Unused vials of MAST SPUTAGEST™ should be stored at 2-8°C and are stable for 12 months from date of manufacture.

Reconstituted vials if kept sterile are stable for up to 48 hours at 2-8°C.

Safety Information

Dithiothreitol may be harmful by ingestion, if inhaled as dust and may by irritating to eyes. Dithiothreitol has an unpleasant odour!

When in use, follow standard microbiological procedures both in the handling of organisms and in disposal of contaminated material.

References