Novobiocin Selectatab™
Novobiocin Selectavial™

MS30, SV30  For the selective isolation of Salmonella spp. by motility enrichment and the selective enrichment of E.coli O157 in food and faeces samples.

Introduction

Conventional procedures for the isolation of salmonellae are time consuming and labour intensive, often requiring the use of several types of media. De Smedt and his colleagues found modified semi-solid Rappaport-Vassiliadis medium (MSRV), including novobiocin to inhibit growth of competing organisms such as Proteus, Escherichia coli and Pseudomonas, was reliable for the isolation of Salmonella spp. from food samples.

The majority of outbreaks and sporadic cases of haemorrhagic colitis (bloody diarrhoea) and haemolytic uraemic syndrome (HUS) in the United Kingdom are associated with E.coli of serogroup O157 (E.coli O157). Outbreaks in the UK have been associated with the consumption of water and various foods, most commonly of bovine origin, including, minced beef, yoghurt and unpasteurised milk, has only rarely been successful. Use of tryptone soy broth, modified by the addition of bile and buffer salts, and Novobiocin as an enrichment medium has proved to be of value in the examination of faeces samples.

Description

MAST has available supplements for the selective enrichment of Salmonella spp. and E.coli O157 - MAST Novobiocin Selectatab™ is designed for direct addition to 100ml of media. MAST Novobiocin Selectavial™ is designed for addition to 2.25 litres of medium after reconstitution with an approved diluent.

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<th>MS30 Novobiocin Selectatab™</th>
<th>SV30 Novobiocin Selectavial™</th>
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<td>Novobiocin</td>
<td>2.0mg</td>
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Directions

1. Motility enrichment of Salmonella spp.

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

2. Sterilise the medium, MAST MSRV (Salmonella) medium (DM440), 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. (For larger quantities reconstitute one vial of SV30 Novobiocin Selectavial™ using 20ml of sterile water and add to 2.25 litres of medium).

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

5. 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 3 drops (approximately 0.1ml each) of an enriched culture in separate spots on the surface of an MSRV plate. Allow the spots to air dry and incubate the plates at 42°C. After 24 hours examine the plates for migration and confirm the identification of suspect colonies as Salmonella by slide agglutination using MAST ASSURE™ Antisera or biochemical methods.

2. Selective enrichment of E.coli O157

Food Samples

1. Sterilise the medium, MAST Modified Tryptone Soy Broth (DM622) cool to 50-55°C and hold in a water bath at this temperature.

2. Reconstitute one SV30 Novobiocin Selectavial using 20ml of sterile water and add 2ml to 225ml of basal medium or one MS30 Novobiocin Selectatab per 100ml.

3. Mix gently, but thoroughly, to evenly distribute the selective agents.
4. Prepare a $10^{-1}$ homogenate of food sample, using either a stomacher or blender, by homogenising 25g of sample in 225ml of broth.

5. Incubate at 42°C for 22 hours, preferably with agitation, and subculture onto plates of CT-SMAC medium (MAST DM491/SV48/SV49) after 6 and 22 ± 2 hours. If immunomagnetic separation techniques are used the broth should be processed after 6 hours incubation

6. Incubate CT-SMAC plates at 37°C for 24 hours and examine for the presence of non-sorbitol fermenting colonies.

7. Subculture five suspect colonies (or all visible colonies if fewer than five) onto plates of MAST MacConkey Agar (DM140) and confirm the serotype of gram negative lactose fermenting bacilli with suitable antisera (MAST ASSURE™ Antisera M12030 for E.coli O157).

Faeces samples

1. Sterilise the medium. MAST Modified Tryptone Soy Broth (DM622) cool to 50-55°C and hold in a water bath at this temperature.

2. Reconstitute one SV30 Novobicin Selectavial using 20ml of sterile water and add to 2.25 litres of medium or one MS30 Novobiocin Selectatab directly to 100ml.

3. Mix gently, but thoroughly, to evenly distribute the selective agents.

4. Aseptically distribute the medium, in 10ml volumes, into previously sterilised containers.

5. Inoculate approximately 0.5g of faeces into 10ml of prepared broth.

6. Incubate at 37°C for 18-22 hours and subculture onto plates of CT-SMAC medium (MAST DM491/SV48/SV49). Incubate the CT-SMAC plates at 37°C for 24 hours, examine for the presence of non sorbitol fermenting colonies and confirm as above.

References


