

## Modified Tryptone Soy Broth

A selective enrichment broth for the recovery of *Escherichia coli* O157:H7 from food or faecal samples.

### 1. Description

The majority of outbreaks and sporadic cases of haemolytic uraemic syndrome (HUS) and haemorrhagic colitis (bloody diarrhoea) in both the United Kingdom and United States are associated with verocytotoxigenic *Escherichia coli* of serogroup O157:H7 (*E.coli* O157:H7)<sup>2</sup>. 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<sup>3</sup> but, isolation from suspect foods, notably red meat, has only rarely been successful<sup>4</sup>.

The recently published report of the Advisory Committee on the Microbiological Safety of Food on verocytotoxigenic producing *E.coli* (VTEC)<sup>5</sup> highlights the deficiencies in methodology for the detection of *E.coli* O157:H7 in food and stresses the requirement for methods to be able to detect potentially very low numbers of organisms which may be present in a food sample.

This is an important consideration since the number of organisms of this serotype required to produce infection is very low and the organism can be transmitted efficiently via contaminated foodstuffs. In a large

outbreak due to *E.coli* O157:H7 in the USA in 1993 an infectious dose of 40 organisms was reported<sup>6</sup>. The suggestion of a low infectious dose for humans is also supported by reports of laboratory acquired infection with strains of *E.coli* O157:H7, particularly since, in these cases, there were no apparent lapses in good laboratory practice<sup>7</sup>.

Recent work within the UK Public Health Laboratory Service (PHLS) to enhance detection of the organism has identified a method utilising tryptone soy broth, modified by the addition of bile and buffer salts, and Novobiocin, as a selective enrichment medium<sup>8</sup>. The MAST formulation (DM622/SV30/MS30) is based on this work and is intended to be used in conjunction with a selective agar medium: CT-SMAC (DM491/SV48/SV49). The selective enrichment medium is also suitable for use with immunomagnetic separation (IMS) procedures prior to plating onto CT-SMAC medium.

About half of all patients infected with *E.coli* O157:H7 do not have blood in their stools and some of these may progress to HUS without developing haemorrhagic colitis. In

consequence, a recent report of the Advisory Committee on the Microbiological Safety of Food<sup>9</sup> has recommended that all diarrhoeal stools are routinely examined for the presence of *E.coli* O157:H7. Rapid establishment of a diagnosis can greatly assist in the management of outbreaks due

to this organism and an enrichment method has also proved to be of value for the examination of faeces samples. In one study<sup>10</sup>, enrichment was shown to increase, by 36%, the isolation rate of *E.coli* O157:H7 in follow up samples and in samples from contacts of existing cases.

## 2. Technical Formula\*

Formula	grams per litre
Casein hydrolysate	17.0
Soy peptone	3.0
Sodium chloride	5.0
D-glucose	2.5
Di-potassium hydrogen phosphate	4.0
Bile Salts No.3	1.5
<b>pH approx. 7.4</b>	

## 3. Directions

### Food samples

1. Suspend by swirling 7.4g of powder in 225ml, or the contents of the sachet in the stated volume of distilled or deionised water and dissolve completely.
2. Autoclave at 121°C (15 p.s.i.) for 15 minutes.
3. Cool to 55°C and hold in a water bath at this temperature.
4. Reconstitute one vial of Novobiocin Selectavial (SV30) by aseptically adding 20ml of sterile water using a sterile needle and syringe. Draw the dissolved supplement up into the syringe and add 2ml per 225ml of medium. Discard the needle into an approved container. Alternatively, add one MS30 Novobiocin Selectatab directly to 100ml of medium.

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

6. Prepare a 10<sup>-1</sup> homogenate of food sample using either a stomacher or blender, by homogenising 25g of sample in 225ml of broth.

7. 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.

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

9. 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™ product M12030 for *E.coli* O157:H7).

## Faeces samples<sup>1</sup>

1. Suspend by swirling 3.3g of powder in 100ml of distilled or deionised water and dissolve completely.
2. Autoclave at 121°C (15 p.s.i.) for 15 minutes.
3. Cool to 55°C. Reconstitute one SV30 Novobio Selectavia using 20ml of sterile water and add to 2.25 litres of medium or one MS30 Novobiocin Selectatab directly to 100ml.
4. Mix well and aseptically distribute the medium 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.

## 4. References

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