

HiCrome™ MacConkey Sorbitol Agar Base

M1340

Intended Use:

Recommended for selective isolation of *Escherichia coli* O157:H7 from food, animal feeding stuffs and clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptone	17.000
Proteose peptone	3.000
Sorbitol	10.000
Bile salts mixture	1.500
Sodium chloride	5.000
Crystal violet	0.001
Neutral red	0.030
B.C. indicator	0.100
Agar	13.500
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 25.06 grams in 495 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. If desired rehydrated contents of one vial of Tellurite-Cefixime Supplement (FD147) may be added aseptically to 495 ml sterile molten, cooled medium before pouring into sterile Petri plates.

Principle And Interpretation

Sorbitol MacConkey Agar is based on the formulation described by Rappaport and Henig (6). The medium contains sorbitol instead of lactose and it is recommended for the detection of enteropathogenic strains of *Escherichia coli* O157:H7 that ferments lactose but does not ferment sorbitol (5) and hence produce colourless colonies. Sorbitol fermenting strains of *Escherichia coli* produce pink-red colonies. The red colour is due to production of acid from sorbitol, absorption of neutral red and a subsequent colour change of the dye when pH of the medium falls below 6.8. *Escherichia coli* O157:H7 has been recognised as a cause of haemorrhagic colitis (4). March and Ratnam (5) reported that the detection of *Escherichia coli* O157:H7 had a sensitivity of 100% and specificity of 85% on Sorbitol MacConkey Agar and they recommended this medium as reliable means of screening *Escherichia coli* O157:H7. B.C. indicator is added to detect the presence of an enzyme β-D-glucuronidase, which is specific for *Escherichia coli* (1). Strains of *Escherichia coli* fermenting sorbitol and possessing β-D-glucuronidase appear as blue - purple coloured colonies on the medium. Enteropathogenic strains of *Escherichia coli* O157:H7 do not possess β-D-glucuronidase activity (8) and do not ferment sorbitol, thus produce colourless colonies.

Tryptone and proteose peptone provide carbonaceous, nitrogenous compounds, long chain amino acids, vitamins and other essential growth nutrients. Most of the gram-positive organisms are inhibited by crystal violet and bile salts. Sodium chloride maintains the osmotic equilibrium.

Addition of Tellurite-Cefixime Supplement makes the medium selective (9). Potassium Tellurite selects the serogroups and inhibits *Aeromonas* species and *Providencia* species Cefixime inhibits *Proteus* species. *Pseudomonas* if present produces colourless colonies on this medium. For confirmation oxidase test may be performed with suspected colonies and results should be noted within 5-10 seconds.

Type of specimen

Clinical samples : stool , Food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3).

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (7).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. β -glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative.

2. Some species may show poor growth due to nutritional variations.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.35% Agar gel.

Colour and Clarity of prepared medium

Purplish red coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.01% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours(48 if necessary)

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony w/o addition of FD147	Oxidase Reaction
<i>Escherichia coli</i> O157:H7 (NCTC 12900)	50-100	good-luxuriant	>=50%	colourless	negative reaction
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	none-poor	<=10%	blue-green	negative reaction
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	50-100	fair-good	30-40%	colourless	positive reaction,deep purple blue colour develops within 10 seconds
<i>Klebsiella pneumoniae</i> ATCC 13883 (00097*)	50-100	none-poor	<=10%	pink-red	negative reaction

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store dehydrated powder and prepared medium on receipt at 2-8°C. Use before expiry period on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from

extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

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5. March S.B. and Ratnam S. (1986): J. Clin. Microbiol. 23,869-872.
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8. Thompson et al (1990). J. Clin. Microbiol. 29, 2165-2168.
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In vitro diagnostic medical device



CE Marking



Storage temperature



Do not use if package is damaged



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