



Technical Data

HiCrome MacConkey Sorbitol Agar Base

M1340

HiCrome MacConkey Sorbitol Agar is recommended for selective isolation of *Escherichia coli* O157:H7 from food and animal feeding stuffs.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	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 50°C. Mix well and pour into sterile Petri plates. If desired rehydrated contents of 1 vial of Tellurite-Cefixime Supplement (FD147) may be added aseptically to 495 ml sterile molten, cooled (50°C) medium before pouring into sterile Petri plates.

Principle And Interpretation

Sorbitol MacConkey Agar is based on the formulation described by Rappaport and Henig (1). 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 (2) 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 (3). March and Ratnam (2) 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*. (4). 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 (5) and do not ferment sorbitol, thus produce colourless colonies.

Casein enzymic hydrolysate and proteose peptone provide carbonaceous, nitrogenous 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 (6). 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.

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	50-100	good	40-50%	blue-green	negative reaction
<i>Pseudomonas aeruginosa</i> ATCC 27853	50-100	fair-good	30-40%	colourless	positive reaction, deep purple blue colour develops within 10 seconds
<i>Klebsiella pneumoniae</i> ATCC 13883	50-100	good	40-50%	pink-red	negative reaction

Storage and Shelf Life

Store dehydrated powder and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

1. Rappaport F. and Henigh E., 1952, J. Clin. Path., 5:361.
2. March S.B. and Ratnam S. (1986): J. Clin. Microbiol. 23,869-872.
3. Karmali M.A., Petric M., Lim C., et al, 1985, J. Infect. Dis. 151-775.
4. Hansen W. and Yourassawsky E., 1984, J. Clin. Microbiol., 20:1177.
5. Thompson et al (1990). J. Clin. Microbiol. 29,2165-2168.
6. Zadik P.M., Chapman P.A., and Siddons C.A. (1993), J. Med. Microbiol. 39, 155-158

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