HiCrome™ MacConkey Sorbitol Agar Base

Recommended for the selective isolation of *Escherichia coli* 0157:H7 from clinical food and animal feeding stuff.

M1340

Composition **

Ingredients	Grams/Litre
Tryptone	17.00
Proteose peptone	3.00
Sorbitol	10.00
Bile salts mixture	1.50
Sodium chloride	5.00
Crystal violet	0.001
Neutral red	0.03
B.C. Indicator	0.10
Agar	13.50

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 1 vial of Tellurite-Cefexime Supplement (FD147) may be added aseptically to 495 ml sterile molten, cooled (45-50°C) medium before pouring into sterile Petri plates.

Principle and Interpretation

Sorbitol MacConkey Agar is based on the formulation described by Rappaport and Henigh (4). 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 hemorrhagic colitis (2). March and Ratnam (3) 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 the 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 (5) and thus produce colourless colonies.

Tryptone 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.

Type of specimen

Clinical, Food and animal feeding stuff, Dairy samples.

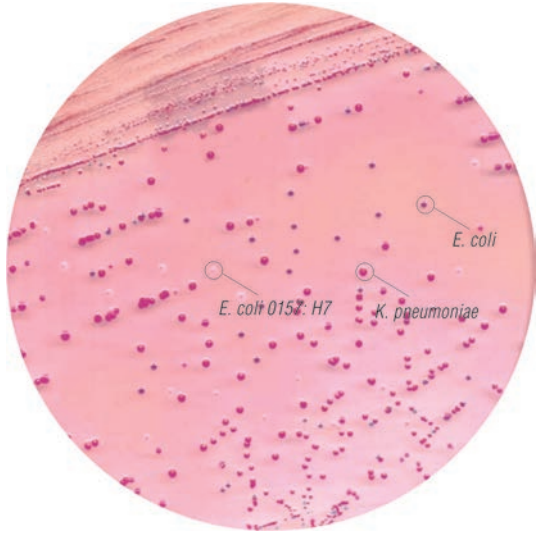
Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (10, 11).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7, 8, 9).

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.



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Limitations

1. *Pseudo*monas 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.
2. Some species may show poor growth due to nutritional requirements.

Performance and Evaluation

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

Quality Control

Appearance of powder : Light yellow to pink coloured, 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.

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

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	#Colour of colony	Oxidase
Escherichia coli O157:H7 (NCTC 12900)	50-100	good - luxuriant	>50%	colourless	-
Escherichia coli (25922) (00013*)	50-100	good	40-50%	blue-green	-
Pseudomonas aeruginosa (27853) (00025)*	50-100	fair-good	30-40%	colourless	+
Klebsiella pneumoniae (13883) (00097)*	50-100	good	40-50%	pink-red	-

Key : # = Colour of the colony without addition of Tellurite-Cefixime Supplement (FD147)
+ = positive reaction deep-purple blue colour develops within 10 seconds
- = negative reaction
* = corresponding WDCM Numbers

Storage and Shelf-life

Store between 2-8°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (10, 11).

References

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