

Technical Information

MacConkey Sorbitol Agar Base

Product Code: DM 1298I

Application: - MacConkey Sorbitol Agar Base is used as a selective medium for isolation and detection of *Escherichia coli* O157:H7 from food and animal feed stuff.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	17.000
Meat peptone	3.000
D-Sorbitol	10.000
Bile salts mixture	1.500
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.001
Agar	13.500
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

MacConkey Sorbitol Agar Base is used by ISO Committee (3) with a slight modification of MacConkey Sorbitol Agar formulated by Rappaport and Henigh (1). This medium is used for isolation of enteropathogenic *Escherichia coli* O157: H7, which ferments lactose but does not ferment sorbitol, hence produces colourless colonies. This organism has been recognized as a cause of hemorrhagic colitis (2). *E.coli* O157: H7 is a human pathogen associated with hemorrhagic colitis that results from the action of a shiga-like toxin (SLT) (5, 6).

MacConkey Sorbitol Agar however should not be solely used to detect pathogenic *E.coli* O157: H7 strains as some non- toxic strains will also not ferment sorbitol (4).

On standard MacConkey Agar containing lactose, this strain is indistinguishable from other lactose-fermenting *E.coli*. In MacConkey Sorbitol Agar Base, lactose is replaced by sorbitol. Unlike most *E.coli* strains, *E.coli* O157:H7 ferments sorbitol slowly or not at all (8, 9). The growth of *E.coli* O157:H7 on MacConkey Agar with Sorbitol shows colourless colonies and most of the fecal flora ferment sorbitol and appear pink. MacConkey Agar with Sorbitol therefore permits ready recognition of *E.coli* O157:H7 (5, 6, 7).

Casein enzymic hydrolysate and meat peptone provide necessary nutrients like nitrogenous and carbonaceous compounds, minerals, vitamins and trace ingredients for the growth of organisms. Crystal violet and bile salt mixture present in the medium prevent growth of gram-positive bacteria. The addition of cefixime and tellurite, as MS 2147 significantly reduces the number of sorbitol non-fermenters that are to be screened during the attempted isolation of *E.coli* O157:H7. Sodium chloride helps to maintain osmotic equilibrium in the medium. Neutral red is an indicator. D-Sorbitol is the fermentable carbohydrate.

Methodology

Suspend 50.03 grams of dehydrated powder media in 990 ml distilled water. Mix thoroughly & heat to boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of 2 vials of Tellurite- Cefixime Supplement (M 2147). Shake well and pour into sterile Petri plates.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.35% Agar gel.

Colour and Clarity

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

Reaction

Reaction of 5% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH Range

6.90-7.30

Cultural Response

DM 1298I: Cultural characteristics observed with added Tellurite - Cefixime Supplement (MS 2147), after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Escherichia coli</i> 0157:H7 NCTC 29900	50-100	good-luxuriant	≥50%	colourless
<i>Escherichia coli</i> ATCC 25922	≥10 ³	inhibited	0%	-
<i>Pseudomonas aeruginosa</i> ATCC 27853	50-100	none-poor	≤10%	colourless
<i>Staphylococcus aureus</i> ATCC 25923	≥10 ³	inhibited	0%	-

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Rappaport F. and Henigh E., 1952, J. Clin. Pathol., 6 : 361.
2. Karmali M. A., Petric M., Lim C. et al, 1985, J. Infect. Dis., 151:775.
3. ISO 1995, Draft ISO/ DIS 16654:1999.
4. Pelczar M. J., Chan E. C. and Kreig M. R., 1986, Microbiology, 5th Ed., McGraw Hill Book Co., New York,
5. March S. B. and Ratnam S., 1986, J. Clin. Microbiol., 23:869.
6. Centre for Diseases Control, 1991, Morbid. Mortal, Weekly Rep 40:265.
7. Murray P. R., Baron J. H., Pfaller M. A., Tenover F. C. and Tenover R. H. (Ed.), 1999, Manual of Clinical Microbiology, 7th Ed. American Society for Microbiology, Washington, D. C.
8. Zadik J. M., Chapman P. A. and Siddons C. A., 1993, J. Med. Microbiol., 39:155.
9. Sanderson M. W., Gay J. M., Hancock D. D., Gay C. C., Fox L. K. and Besser T. E., 1955, J. Clin. Microbiol., 33: 2616.

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