MacConkey Sorbitol Agar Base

M298I

Intended Use:

Recommended as the selective medium for isolation and detection of *Escherichia coli* O157:H7 from food, animal feeding stuffs and clinical samples. The composition and performance criteria are in accordance with ISO 16654:2001& / Amd 2:2023.

Composition**

As per ISO 16654:2001 Amd 2:2023

Ingredients	g / L
Enzymatic digest of casein	17.000
Enzymatic digest of animal tissues	3.000
Sorbitol	10.000
Bile salts No.3	1.500
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.001
Agar	9.0-18.0
Final pH (at 25°C)	7.1±0.2

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Ingredients	g / L
Tryptone$	17.000
HM 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

Supplement to be added after sterilization

*Ingredients	Concentration
Potassium tellurite	2.500mg
Cefixime	0.050mg

TC Selective Supplement (FD147) - 2 vials

*Ingredients	Concentration
Potassium tellurite	1.250mg
Cefixime	0.025mg

**Formula adjusted, standardized to suit performance parameters

$- Equivalent to Enzymatic digest of casein ; # Equivalent to Enzymatic digest of animal tissues

Directions

Suspend 50.03 gram in 990 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. **AVOID OVERHEATING.** Cool to 45-50°C and aseptically add rehydrated contents of 2 vials of TC Selective Supplement (FD147). Mix well and pour into sterile Petri plates.

Principle And Interpretation

MacConkey Sorbitol Agar is recommended by ISO Committee (1) with a slight modification of MacConkey Sorbitol Agar formulated by Rappaport and Henigh (2). This medium is recommended 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 (3). *E.coli* O157: H7 is a human pathogen associated with hemorrhagic colitis that results from the action of a shiga-like toxin (SLT) (4,5). 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 (6).

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 (7,8). The growth of *E.coli* O157:H7 on MacConkey Agar with Sorbitol shows colourless colonies and most of the faecal flora ferment sorbitol and appear pink. MacConkey Agar with Sorbitol therefore permits ready recognition of *E.coli* O157:H7 (4,5,9).

Tryptone and HM peptone supply 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 inhibit growth of gram-positive bacteria. The addition of cefixime and tellurite, as FD147 significantly reduces the number of sorbitol non-fermenters that are to be screened during the attempted isolation of *E.coli* O157:H7. Sodium chloride maintains osmotic equilibrium. Neutral red is an indicator. D-Sorbitol is the fermentable carbohydrate.

Type of specimen

Food samples

Specimen Collection and Handling:

ISO 16654:2001&/Amd 2:2023 and ISO 11133:2014 /Amd. 2 : 2020 (E) (1,10)

a) Enrichment of the test portion homogenized in Modified Soyabean Bile Broth Base (M1286I) with incubation at 41.5±1 °C for 6 h and subsequently for a further 12 h to 18h.

b) Isolation onto MacConkey Sorbitol Agar Base (M298I). Incubate at 37±1°C for 21±3 h. Confirm the colonies by biochemical characterisation.

Warning and Precautions :

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further subculture must be carried out for confirmation.

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

pH

6.90-7.30

Cultural Response :

Productivity : Cultural characteristics observed with added TC Selective Supplement (FD147) , after an incubation at 36-38°C for 18-24 hours.

Selectivity : Cultural characteristics observed with added TC Selective Supplement (FD147) , after an incubation at 36-38° C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Characteristic reaction
Productivity			
Escherichia coli O157:H7 ATCC 700728 (00014*)	10^3 -10^4	Good growth	Transparent colonies with a pale yellowish-brown appearance
Selectivity			
Escherichia coli ATCC 25922 (00013*)	>=10^4	Partial inhibition w/ growth of some pink colonies	
Escherichia coli ATCC 8739 (00012*)	>=10^4	Partial inhibition w/ growth of some pink colonies	
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	Inhibited	
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10^4	Inhibited	

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°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 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. 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 (11,12).

Reference

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