

MacConkey Sorbitol Agar Plate

MP298I

Intended Use:

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

Composition**

As per ISO 16654:2001 Amd 2:2023

Ingredients	Gms / Litre
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
Potassium tellurite (0.25%)	1.00ml
Cefixime (0.5%)	1.00ml
Agar	9.0-18.0
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters # Equivalent to Meat peptone

MacConkey Sorbitol Agar plate MP298I

Ingredients	Gms / Litre
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
TC Selective Supplement (FD147)	2 vial
Potassium tellurite	1.250mg
Cefixime	0.025mg
Agar	13.500
Final pH (at 25°C)	7.1±0.2

Directions

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

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 fecal 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

Water samples

Specimen Collection and Handling:

ISO 16654:2001&/Amd 2:2023 (1)

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.

a) Isolation onto MacConkey Sorbitol Agar Base (M298I). Incubate at 37±1°C for 21±3h. 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

Sterile MacConkey Sorbitol Agar Plate in 90 mm disposable plates with smooth surface and absence of black particles/ cracks/bubbles

Colour of medium

Purplish red coloured medium

Quantity of medium

25 ml of medium in 90 mm disposable plates.

Sterility Check

Passes release criteria

pH

6.90-7.30

Cultural Response

Cultural characteristics observed with after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	
Productivity			
<i>Escherichia coli</i> O157:H7 NCTC 12900	50-100	>10	Characteristic colonies on M298I
+ <i>Staphylococcus aureus</i> <i>subsp. aureus</i> ATCC 25923 (00034*)			
+ <i>Staphylococcus aureus</i> <i>subsp. aureus</i> ATCC 6538 (00032*)			
Selectivity			
<i>Staphylococcus aureus</i> <i>subsp. aureus</i> ATCC 25923 (00034*)	≥10 ⁴	Total inhibition on M290	
<i>Staphylococcus aureus</i> <i>subsp. aureus</i> ATCC 6538 (00032*)	≥10 ⁴	Total inhibition on M290	
Productivity			
<i>Escherichia coli</i> O157:H7 NCTC 12900	50-100	Good	Transparent colonies with a pale yellowish-brown appearance
<i>Staphylococcus aureus</i> <i>subsp. aureus</i> ATCC 25923 (00034*)	≥10 ⁴	Total inhibition	
<i>Staphylococcus aureus</i> <i>subsp. aureus</i> ATCC 6538 (00032*)	≥10 ⁴	Total inhibition	

<i>Escherichia coli</i> ATCC 25922 (00013*)	$\geq 10^4$	Partial inhibition w/ growth of some pink colonies
<i>Escherichia coli</i> ATCC 8097 (00012*)	$\geq 10^4$	Partial inhibition w/ growth of some pink colonies

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

On receipt store between 20-30°C. 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).

Reference

1. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Escherichia coli* O157 AMENDMENT 2: Inclusion of performance testing of all culture media and reagents, ISO 16654:2001, Amd 2:2023.
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