

Instructions for Use

Sorbitol MacConkey Agar

Pre-Poured Media

REF - PP0250

1. Intended Use

For *in vitro* diagnostic use. PP0250 Sorbitol MacConkey Agar is a differential medium used in the isolation of *Escherichia coli* O157:H7.

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
Peptone	20.0
Sorbitol	10.0
Bile salts	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	13.5

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

The serotype O157 of *Escherichia coli* is a pathogenic serotype, identified for the first time in 1982 as the agent responsible for haemorrhagic colitis. Animal origin food is the principal source of human contamination. Beef and pork tissue, poultry, and certain non-pasteurized dairy products have been implicated in infections linked to *E. coli* O157. This microorganism has also been isolated from potatoes, apple cider and tap water.

Sorbitol MacConkey Agar is based on the formulation by Rappaport and Henig ⁽¹⁾. In 2009, the Centres for Disease Control and Prevention (CDC) issued guidelines for clinical laboratories with the recommendation that all stools submitted for routine testing from patients with acute community-acquired diarrhoea should be cultured for *E. coli* (STEC) O157:H7.

4. Principle

Peptone provides a source of nitrogen and other essential nutrients. Sorbitol is a differentially fermented sugar. *E. coli* O157 does not ferment this sugar to produce acid so grows as colourless colonies. Most other strains will ferment sorbitol and the resultant acid production reduces pH and changes the colour of the neutral red indicator system to produce pink colonies. Some sorbitol positive organisms may lose the ability to ferment sorbitol and produce colourless colonies giving a false negative result⁽³⁾. Bile salts and crystal violet are selective agents and together inhibit Gram positive organisms.

5. Physical Characteristics

	Medium
Appearance and Colour	Pale red-violet tinge
pH	7.1 ± 0.2

6. Materials Provided

PP0250 Sorbitol MacConkey Agar is supplied in packs of 10 x 90mm P090 Petri dishes (18-22ml fill) (product code: PP0250 -P090); each plate is ink-jet printed with (abbreviated) product name, product code, lot number and expiry date.

7. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., autoclave, sterile loops or swabs, collection containers, incubators, and quality control organisms.

8. Specimens

PP0250 Sorbitol MacConkey Agar is suitable for the testing of the following specimens:

- Food Industry: products for human consumption, animal feed and environmental samples
- Clinical: rectal swabs and stool specimens

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

9. Test Procedures and Interpretation of results

Based on sample type and information provided, determine whether the specimen requires pre-enrichment prior to inoculation to the prepared medium.

Food and Environmental Samples:

Prepare a homogenised suspension using a ratio of 1:10 of sample to Maximum Recovery Diluent (E&O BM0760). Plate directly onto agar and incubate at $37 \pm 1^\circ\text{C}$ aerobically for 16-24 hours.

Clinical Samples:

Inoculate specimen directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker. Incubate at $37 \pm 1^\circ\text{C}$ aerobically for 16-24 hours.

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical, serological, molecular, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

10. Quality Control

Organism	Incubation	Result (Specificity)
<i>E. coli</i> O157 (NCTC 12900)	$37 \pm 1^\circ\text{C}$ aerobically for 16-24 hours	Growth: Translucent colonies
<i>E. coli</i> (NCTC 12241)	$37 \pm 1^\circ\text{C}$ aerobically for 16-24 hours	Growth: Pink colonies
<i>S. aureus</i> (NCTC 12981)	$37 \pm 1^\circ\text{C}$ aerobically for 16-24 hours	Inhibited

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

11. Performance

To fully verify PP0250 Sorbitol MacConkey Agar performance, samples were tested to assess colony morphology and recovery level (where an acceptable range is $\geq 50\%$ and $\leq 120\%$) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu for the target organisms and 10^4 - 10^5 cfu for the non-target organisms then incubated at $37 \pm 1^\circ\text{C}$ aerobically for 16-24 hours. All samples showed good recovery and the correct morphology of the required test organisms: *Escherichia coli* O157 (NCTC 12900) and *Escherichia coli* (NCTC 12241) and no recovery of the non-target test organism: *Staphylococcus aureus* (NCTC 12981). Therefore, it can be concluded that PP0250 Sorbitol MacConkey Agar, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. Limitations of the Medium

- Prepared Sorbitol MacConkey medium may grow bacteria other than *E. coli*.
- Some non-O157 serotypes may lose the ability to ferment sorbitol thus producing a false positive result.
- Due to natural variation, some strains may grow poorly on this medium.
- Incubation for more than 24 hours may result in changes to typical appearance of *E. coli* O157 colonies ⁽²⁾
- Some strains of *Proteus* may swarm on this medium

13. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to E&O Poured Plate Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store PP0250 in the original packaging between 2 and 8°C in the dark. Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements. Do not use medium if it is contaminated, discoloured, out of date, cracked or a precipitate is present. Additionally, do not use medium if it has been stored inappropriately or the packaging has been damaged.

15. References

1. Rappaport, F. and Henig, E. (1952) Media for the Isolation and Differentiation of Pathogenic Bact. coli (Serotypes O111 and O55). J. Clin. Pathol., 5(4), 361-362.
2. Adams, S. (1991) Screening for verotoxin-producing *Escherichia coli*. Clin. Lab. Sci., 4, 19-20.
3. MacConkey, A. (1908) Bile Salt Media and their advantages in some Bacteriological Examinations. Am. J. Hyg., 8(3), 322-34.

Version History*

001 18/10/23 - New Document Created

*Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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TABLE OF APPLICABLE SYMBOLS

IFU/PP0250 REV. 001

REF o REF Catalogue number	LOT Batch code	IVD In vitro Diagnostic Medical Device	 Manufacturer	 Use by
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Keep away from direct light	 Store in a dry place