Instructions for Use

REF - PP1940 Sorbitol MacConkey with Cefixime and Tellurite (CT-SMAC)

Pre-Poured Media

1. Intended Use

For *in vitro* diagnostic use. PP1940 Sorbitol MacConkey with Cefixime and Tellurite (CT-SMAC) is a selective differential medium for the isolation of Shiga toxin-producing *Escherichia coli* (STEC) serotype O157:H7 from faecal, food and environmental specimens.

2. Composition*

Ingredient	g/L
Peptone	20.0
Cefixime	0.00005
Sorbitol	10.0
Potassium tellurite	0.0025
Bile salts No.3	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. Food of animal origin is the principal source of human contamination. Beef, pork, poultry, and certain non-pasteurized dairy products have been implicated in infections caused by *E. coli* O157. This microorganism has also been isolated from potatoes, apple cider and tap water. CT-SMAC is recommended by the ISO 16654 [1] for the testing of food and environmental samples and tested in accordance with ISO 11133:2014 [2]. It is also recommended in Public Health England's ID22 [3] and S7 [4] methods.

CT-SMAC is based on the formulation by Rappaport and Henig [5]. In 2009, the Centers 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* O157:H7 (STEC). UK Standards for Microbiological Investigations S7 [4] states that all diarrhoeal samples with obvious blood along with those from children under five or adults over 65 should be routinely tested for the presence of *E. coli* O157.

4. Principle

In this medium, sorbitol has replaced lactose as the main source of fermentable carbohydrate. Since *E. coli* 0157:H7 does not produce acid from sorbitol, it grows as pale translucent colonies; whereas most other strains of *E. coli* are sorbitol positive and produce pink colonies (it should be noted that some sorbitol positive organisms may revert to translucent colonies and produce a false positive [6 4]). Tryptone and meat peptone provide carbon, nitrogen and vitamins, and sodium chloride maintains the osmotic balance. Sorbitol fermentation by non-O157 strains of *E. coli* produces acid and turns the neutral red indicator pink [4]. Bile salts and crystal violet are selective agents and together inhibit Gram-positive organisms.

Cefixime is a broad-spectrum cephalosporin antibiotic effective against a wide range of Gram-positive and Gram-negative bacteria. It acts to inhibit cell wall synthesis. *E. coli* O157 produces extended spectrum β-lactamase activity that inactivates cephalosporins and renders the organism resistant to these antibiotics [7].

Potassium tellurite is a selective agent with inhibitory action against both Gram-positive and Gram-negative bacteria. It generates inhibitory oxidative stress and leads to production of reactive oxygen species within susceptible organisms. *E. coli* O157 and other non-O157 shigella-toxin producing strains are able to reduce tellurite to tellurium thus negating tellurite toxicity [2].

5. Physical Characteristics

	Medium
Appearance and Colour	Pale red firm gel with a violet tinge
pH	7.1 ± 0.2

6. Materials Provided

PP1940 Sorbitol MacConkey with Cefixime and Tellurite (CT-SMAC) is supplied in packs of 10 x 90mm P090 Petri dishes (18-22ml fill) (product code: PP1940-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., sterile loops or swabs, collection containers, incubators, and quality control organisms.

8. Specimens

PP1940 Sorbitol MacConkey with Cefixime and Tellurite (CT-SMAC) is suitable for the testing of the following specimens:

- Food and Environmental: products for human consumption, animal feed and environmental samples
- Clinical Specimens: 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

Allow the plates to come to room temperature.

Food and Environmental Specimens [(1)]:

Prepare a homogenised suspension using a ratio of 1:10 of sample to pre-warmed Modified Tryptone Soya Broth (E&O BM2130). Incubate at 41.5 ± 1°C for 6 hours and subsequently for a further 12 to 18 hours to give a total incubation period of 18-24 hours.

Perform immunomagnetic separation after the initial 6-hour incubation period and again after the second incubation period prior to plating onto selective agar.

Inoculate 0.05ml of washed and re-suspended IMS particles to CT-SMAC agar plate and streak for isolated colonies. Incubate aerobically at 37 ± 1°C for 18-24 hours.

Clinical Specimens:

All diarrhoeal specimens with obvious blood and all specimens from children under 5 years old should be pre-enriched prior to culture. Specimens should be pre-enriched in Modified Tryptone Soya Broth (E&O BM2130) and incubated aerobically at 35-37°C for 16-24 hours.

Inoculate specimen, or pre-enriched specimen, directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker. Incubate inoculated plates aerobically at 35-37°C for 16-24 hours [(4)].

After incubation, examine agar for typical colourless, non-sorbitol fermenting colonies. 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)
E. coli O157 (NCTC 12900)	37 ± 1°C for 16-24 hours	Growth: Pale colourless colonies
E. coli (NCTC 12241)	37 ± 1°C for 16-24 hours	Inhibited
S. aureus (NCTC 12981)	37 ± 1°C 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 PP1940 Sorbitol MacConkey with Cefixime and Tellurite (CT-SMAC) performance, samples were tested to assess colony morphology and recovery level (where an acceptable range is ≥50% and ≤120%) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu and incubated at 37 ± 1°C for 16-24 hours. All samples showed expected recovery and morphology of the required test organism: *Escherichia coli* O157 (NCTC 12900). Product selectivity was evaluated by inoculating the test plates with ≥3.0 x 10^4 - ≤1.5x10^5 cfu of the required non-target test organisms: Escherichia coli (NCTC 12241) and Staphylococcus aureus (NCTC 12981), which were inhibited. Therefore, it can be concluded that PP1940 Sorbitol MacConkey with Cefixime and Tellurite (CT-SMAC), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. Limitations of the Media

- Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
- Some non-STEC *E. coli* may produce low numbers of small colonies, especially at very high inoculation levels. The resultant colonies are easily distinguishable from O157 strains by their small size and red colour.
- Perform further biochemical, serological, molecular, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to standard method or relevant guidelines.

13. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSSH 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 PP1940 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 media if it is contaminated, discoloured, out of date, cracked or a precipitate is present. Additionally, do not use media if it has been stored inappropriately or the packaging has been damaged.

15. References

1. International Organization for Standardization (2001) ISO 16654:2001. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Escherichia coli* O157. Geneva: ISO.
2. International Organization for Standardization (2014) 11133:2014. Microbiology of food, animal feed and water - Preparation, production, storage, and performance testing of culture media. Geneva: ISO.
3. Public Health England (2015) Identification of Vero cytotoxin-producing Escherichia coli including Escherichia coli O157. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 22(4). London: Standards Unit, Microbiology Services.
4. Public Health England (2020) Gastroenteritis. UK Standards for Microbiology Investigations. Syndromic, S 7(2). London: Standards Unit, National Infection Service.
5. 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.
6. Chapman, P., Siddons, C., Zadik, P. and Jewes, L. (1991) An improved selective medium for the isolation of Escherichia coli O157. J. Med. Microbiol. 35, pp.107-110.
7. Bisi-Johnson, M.A., et al (2023). Isolation and genotypic characterisation of extended-spectrum bets-latamase-producing *Escherichia coli* O157:H7 and *Aeromonas hydrophila* from selected fresh water sources in Southwest Nigeria. Nature Scientific Reports, 13, 10746.

Version History*

001 12/07/23 - Instructions for Use, up to date to current standards. To replace out of date E&O/TDS/PP1940
002 28/08/23 - Added references to ISO and SMIs.

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

EC REP

E&O Laboratories Ltd, Burnhouse, Bonnybridge, Scotland, FK4 2HH, Tel: +44 (0) 1324 840 404, info@eolabs.com, www.eolabs.com

Syntec Scientific Ltd., Unit 2 The Business Centre, Northwest logistics Park, Ballycoolin, Dublin 15, Ireland D1SPY00

TABLE OF APPLICABLE SYMBOLS

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