Reference: 100683AA **Technical Data Sheet**

Product: **CT-SMAC Agar**

Specification

A selective and differential medium for the detection, isolation and enumeration of *E. coli* serotype 0157:H7 formulated according to ISO 16654:2001.

Presentation

	Packaging Details	Shelf Life	Storage
20 Prepared Plates 90 mm with: 21 ± 2 ml	1 box with 2 packs of 10 plates/pack. Single cellophane..	3 months	2-14 ºC

Composition

Composition (g/l):

Casein Peptone	17.00000
Meat Peptone	3.00000
Sorbitol	10.00000
Bile Salts	1.50000
Sodium chloride	5.00000
Crystal violet	0.00100
Neutral red	0.03000
Agar	13.50000
Cefixime	0.00005
Potassium tellurite	0.00250

Description /Technique

Description

The substitution of lactose by sorbitol for the isolation of enteropathogenic Escherichia coli serotypes O111 and O55 was proposed in 1952 by Rappaport and Hening. The usefulness of the medium was shown by March and Ratman (1986) and Adams (1991) for the detection, differentiation and isolation of enterohaemorrhagic (EHEC) and the verotoxin-producing (VTEC) strains of serotype O157:H7 E. coli.

The only modification of the typical MacConkey Medium formulation is the replacement of lactose with sorbitol. The enterohaemorrhagic strains do not use this substrate and produce colourless colonies. The other serotypes can ferment sorbitol and hence produce red colonies.

In all others aspects, MacConkey Agar with sorbitol works similarly to other media in the MacConkey group. Peptone supplies the nitrogen and sodium chloride provides an osmotic environment. Crystal violet and bile salts inhibit the growth of Gram positive bacteria and neutral red acts as the pH indicator.

The sensitivity of SMAC is limited by the difficulty in identifying non-sorbitol-fermenting colonies amongst the accompanying microflora and the possible presence of other non-sorbitol-fermenters such Proteus spp., Aeromonas spp. and some other E. coli which make it necessary to test colonies for confirmation.

Zadik et al. (1993) reported a further improvement in EHEC O157 isolation rates by using SMAC supplemented with cefixime and potassium tellurite (CT-SMAC). Cefixime inhibits Proteus at a concentration not inhibitory to Escherichia coli. EHEC O157 strains are generally less susceptible to tellurite than many other non-sorbitol-fermenters such as Aeromonas spp., Plesiomonas spp., Morganella spp., Providencia spp. and most other E. coli strains. The use of cefixime and tellurite in Sorbitol MacConkey Agar (CT-SMAC) for isolation of E. coli O157:H7 is described in the FDA Bacteriological Analytical Manual and ISO adopted it as the preferred selective medium in its 16654:2001 Standard.

Technique

For plate inoculation follow the laboratories standard methods or the applicable norms (spiral plating method, econometric methods, streak plating, dilution banks, spread plating with drigralsky rod etc ...)

Spread the inoculum onto the dry surface of the medium and incubate at 37 ± 1ºC for 21±3 h. Usually, the O157:H7 serotype forms colourless colonies and the other strains of E. coli produce red colonies. The results must be recorded at 24 hours because an extended incubation produces a decreasing colouration in the sorbitol-fermenting colonies and sometimes a colony of the O157:H7 serotype may begin to ferment sorbitol.

Some Gram negative bacteria such as Pseudomonas, Proteus and Klebsiella can growth on MacConkey Agar with Sorbitol but their colonies are diverse and easy to differentiate from E. coli.

Because of the failure to ferment sorbitol by some strains of no-enterotoxigenic E. coli and atypical colony production by some enterohaemorrhagic strains, the use of other media concurrently with MacConkey Agar with Sorbitol or CT-SMAC is recommended. Confirmation of suspect colonies by serological, biochemical or molecular techniques is also necessary.

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Quality control

Physical/Chemical control

Color : Violet-pink pH: 7.1 ± 0.2 at 25ºC

Microbiological control

Inoculate: Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/ 10^4-10^6 (selectivity).

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at 37 ± 1 ºC, reading after 21 ± 3 h

Microorganism	Growth
E. coli 0157:H7 (non toxg.) ATCC® 700728 WDCM 00014	Good. Colourless colonies (brownish-yellow)
Staphylococcus aureus ATCC® 6538, WDCM 00032	Inhibited
Escherichia coli ATCC® 43888	Poor to good. Transparent colonies (Brown-yellowish)
Escherichia coli ATCC® 8739, WDCM 00012	Partial Inhibition- Red colonies
Stph. aureus ATCC® 25923, WDCM 00034	Inhibited

Sterility Control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

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Storage

Storage conditions: 2-14ºC
Alternatively the plates may also be stored at the range of 2 - 25ºC, with a proper performance of the medium, but some precautions must be taken into account:
-In the range of 2 - 8 ºC avoid direct contact with surfaces that can freeze product.
-In the range of 15 - 25 ºC, dehydration control must be taking in account.