

# MacConkey Agar with Sorbitol (CT-SMAC) ISO

Cat. 1099

Selective and differential medium for Escherichia coli O157:H7

## Practical information

| Applications | Categories            |
|--------------|-----------------------|
| Detection    | Escherichia coli O157 |

Industry: Clinical / Food

Regulations: ISO 11133 / ISO 16654



## Principles and uses

MacConkey Agar with Sorbitol (CT-SMAC) is based on the formula developed by Rappaport & Hening. This medium is recommended for the research of E. coli O157:H7 in clinical and food testing. The composition is similar to MacConkey Agar (Cat. 1052) but the lactose has been substituted with sorbitol for differentiating enteropathogenic E. coli serotypes. These strains are typically sorbitol-negative. On standard MacConkey Agar containing lactose, this strain cannot be distinguished from other lactose fermenting E. coli.

Gelatin peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Sorbitol is the carbohydrate energy source. Bile salts N°3 and Crystal violet are inhibitors of Gram-positive organisms. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Neutral red is the pH indicator, when sorbitol is fermented, the pH of the medium decreases, changing the color from neutral red to pink. Bacteriological agar is the solidifying agent.

E. coli O157:H7 does not ferment Sorbitol and therefore produces colorless colonies. As most of the other E. coli do ferment it, their colonies are pink.

E. coli O157:H7 has become a widespread public health issue as it is responsible for hemorrhagic colitis, characterized by a bleeding diarrhea with acute abdominal pain. Incorrect antibiotic treatment may increase the risk of haemolytic uraemic syndrome development, a potentially fatal complication of this form of colitis.

## Formula in g/L

|                            |      |                                    |       |
|----------------------------|------|------------------------------------|-------|
| Enzymatic digest of casein | 17   | Bacteriological agar               | 15    |
| Bile salts N° 3            | 1,5  | Crystal violet                     | 0,001 |
| Neutral red                | 0,03 | Sodium chloride                    | 5     |
| Sorbitol                   | 10   | Enzymatic digest of animal tissues | 3     |

## Preparation

Suspend 51,5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 44-47 °C, and, if desired, aseptically add two vials of Cefixime Tellurite Supplement (Cat. 6064), previously reconstituted in 5 ml of sterile distilled water. Mix well and dispense into plates.

## Instructions for use

- » For clinical diagnosis, the type of sample is feces.
- Inoculate on the surface. Parallel striae with the handle or hyssop.
- Incubate in aerobic conditions at 35±2 °C for 18-24 hours.
- Reading and interpretation of the results.

» For other uses not covered by the CE marking:

Detection of Escherichia coli O157 according to ISO 16654:

- Prepare the initial suspension adding the test portion to Trypticasein Soy Broth Modified with Novobiacin (Cat. 1292) prewarmed to 41,5 °C to obtain a ratio of 1/10.
- Separate and concentrate the microorganisms by immunogenic particles coated with antibodies to E. coli O157.
- Incubate for 6 hours then a further 12 hours to 18 hours at 41,5 °C.

- Subculture the immunomagnetic particles with the bacteria adhered on MacConkey Agar with Sorbitol (Cat. 1099) and a second selective isolation agar of choice by the laboratory. The optimal incubation temperature for E. coli O157 is 37±1 °C for 18-24 hours.
- Confirm by indol production in Tryptophan Culture Broth (Cat. 1237) and agglutination with the serum anti E. coli O157.

## Quality control

| Solubility | Appearance  | Color of the dehydrated medium | Color of the prepared medium | Final pH (25°C) |
|------------|-------------|--------------------------------|------------------------------|-----------------|
| w/o rests  | Fine Powder | Beige pink                     | Violet red                   | 7,1±0,2         |

## Microbiological test

According to ISO 11133:

Incubation conditions: (37±1 °C / 21±3 h).

Inoculation conditions: Productivity qualitative (10<sup>3</sup> - 10<sup>4</sup> CFU) / Selectivity (10<sup>4</sup>- 10<sup>6</sup> CFU).

| Microorganisms                       | Specification          | Characteristic reaction  |
|--------------------------------------|------------------------|--|
| Escherichia coli ATCC 25922          | Inhibición parcial (1) | Growth of some pink colonies   |
| Staphylococcus aureus ATCC 25923     | Total inhibition (0)   |  |
| Escherichia coli O157:H7 ATCC 700728 | Good growth (2)        | Transparent colonies with a pale yellowish-brown appearance and a diameter ~1 mm |

## Storage

Temp. Min.: 2 °C

Temp. Max.: 25 °C

## Bibliography

Rappaport F. and Hening E. (1952), J.Clin.Path, 5.361. Karmali M.A. (1988), Culture, 9,2. Doyle M.P. and Schoeni S.L (1984), Appl. and Envir. Microbiol., 48, 855-856.

ISO 16654 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Escherichia coli O157.