

MacConkey Sorbitol HiCynth™ Agar (Sorbitol HiCynth™ Agar) MCD298

Intended Use:

Recommended for isolation and identification of enteropathogenic *Escherichia coli* strains associated with infant diarrhea.

Composition**

| Ingredients | g/ L |
|---------------------------|---------|
| HiCynth™ peptone No.3* | 17.000 |
| D-Sorbitol | 10.000 |
| Synthetic detergent No. 1 | 1.500 |
| Sodium chloride | 5.000 |
| Neutral red | 0.030 |
| Crystal violet | 0.001 |
| Agar | 13.500 |
| Final pH (at 25°C) | 7.1±0.2 |

**Formula adjusted, standardized to suit performance parameters

*chemically defined peptones

Directions

Suspend 50.03 gram in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. **AVOID OVERHEATING**. Cool to 45-50°C and pour into sterile Petri plates

Principle And Interpretation

MacConkey Sorbitol HiCynth™ Agar is based on the formulation described by Rappaport and Henigh (1). This medium is recommended for isolation of enteropathogenic *Escherichia coli* O157: H7, which ferments lactose but does not ferment sorbitol, hence produces colourless colonies. MacConkey Sorbitol HiCynth™ Agar is prepared by completely replacing animal peptones with chemically defined peptones to avoid BSE/TSE/GMO risks associated with animal peptone. This organism has been recognized as a cause of hemorrhagic colitis (2). *E.coli* O157: H7 is a human pathogen associated with hemorrhagic colitis that results from the action of a shiga-like toxin (SLT) (3,4).

On standard MacConkey Agar containing lactose, this strain is indistinguishable from other lactose-fermenting *E.coli*. In MacConkey Sorbitol HiCynth™ Agar, lactose is replaced by sorbitol. Unlike most *E.coli* strains, *E.coli* O157:H7 ferments sorbitol slowly or not at all (5,6). The growth of *E.coli* O157:H7 on MacConkey Agar with Sorbitol shows colourless colonies and most of the faecal flora ferment sorbitol and appear pink. MacConkey Agar with Sorbitol therefore permits ready recognition of *E.coli* O157:H7 (3,4,7).

HiCynth™ peptone No.3 supply necessary nutrients like nitrogenous and carbonaceous compounds, long chain amino acids, minerals, vitamins and trace ingredients for the growth of organisms. Crystal violet and synthetic detergent No.1 present in the medium inhibit growth of gram-positive bacteria. Sodium chloride maintains osmotic equilibrium. Neutral red is an indicator. D-Sorbitol is the fermentable carbohydrate.

Type of specimen

Food and Dairy samples. Clinical samples - faeces

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (8,9,10). After use, contaminated materials must be sterilized by autoclaving before discarding.

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. MacConkey Sorbitol HiCynth™ 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).
2. Further biochemical tests must be carried out for further 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

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.35% Agar gel.

Colour and Clarity of prepared medium

Purplish red coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.0% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

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

| Organism | Inoculum (CFU) | Growth | Recovery | Colour of colony |
|---|----------------|-----------|----------|------------------|
| <i>Shigella flexneri</i> ATCC 12022 (00126*) | 50-100 | luxuriant | ≥50% | colourless |
| <i>Escherichia coli</i> ATCC 25922 (00013*) | 50-100 | luxuriant | ≥50% | pink |
| <i>Escherichia coli serotype</i> O11 and O55 | 50-100 | luxuriant | ≥50% | colourless |
| <i>Escherichia coli</i> O157:H7 NCTC 29900 | 50-100 | luxuriant | ≥50% | colourless |

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. 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 (5,11).

Reference

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