HiCrome™ EC O157 : H7 Selective Agar Base, Modified M1575A

Intended Use:

Recommended for selective isolation and easy detection of *Escherichia coli* O157:H7 from food samples.

Composition**

Ingredients	g / L
Tryptone	5.000
Yeast extract	3.000
Sorbitol	7.000
Bile salts mixture	1.500
Sodium lauryl sulphate (SLS)	0.100
Chromogenic mixture	0.250
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.85 gram in 990 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Add rehydrated contents of 1 vial of NP Selective Supplement (FD187) aseptically. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Enterohaemorragic *E.coli* strains are also termed as verocytotoxin-producing *E.coli* (VTEC/ EHEC). Although many different serotypes of *Escherichia coli* are known to produce verocytotoxin (1) those of Escherichia coli O157:H7 and O157:H are so far the common types causing human infections. O157 VTEC strains have several unusual biochemical characters that are exploited in methods for their laboratory identification. They belong to the minority of *E.coli* that are ß-glucuronidase negative and do not ferment sorbitol or rhamnose within 24 hours. These can be isolated from faecal specimens by plating on media containing D-sorbitol instead of lactose. HiCrome™ EC O157:H7 Agar is based on the formulation described by Rappaport and Henigh (2). The medium contains sorbitol as fermentable carbohydrate and chromogenic mixture instead of lactose and indicator dyes respectively The chromogenic substrate is specifically and selectively cleaved by a dark purple to magenta coloured moiety. *E.coli* forms *Escherichia coli* O157:H7 resulting in a dark purple to magenta coloured moiety. *E.coli* forms bluish green coloured colonies.

Tryptone and yeast extract provides carbonaceous and nitrogenous compounds,long chain amino acids,vitamins and growth nutrients. Sodium chloride maintains osmotic equilibrium. Addition of NP Selective Supplement (FD187) makes the medium selective (3). Potassium tellurite selectively inhibits *Aeromonas* and *Providencia* species. Novobiocin inhibits gram-positive bacteria. Sodium lauryl sulphate helps to inhibit the accompanying gram-positive flora.

Type of specimen

Food samples.

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (4).
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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Further biochemical and serological test are necessary for 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

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.18% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed with added NP Selective Supplement (FD187) after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli ATCC 25922 (00013*)	50-100	none to poor	<=10%	Bluish green
Escherichia coli O157:H7 NCTC 12900	50-100	luxuriant	>=50%	dark purple-magenta
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	fair-good	30-40%	colourless-mauve(mucoid)
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	fair to good	30-40%	colourless
Staphylococcus aureus subsp.*aureus* ATCC 25923 (00034*)	>=10^4	Inhibited	0%	
****Bacillus spizizenii*** ATCC 6633 (00003*)	>=10^4	Inhibited	0%	

Key : (*) Corresponding WDCM number and **Formerly known as *Bacillus subtilis* subsp. *spizizenii*

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°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 (5,6).

Reference

1. Smith and Scottland, 1988, J. Med. Microbiol., 26:77-85.
2. Rappaport F. and Henigh E., 1952, J. Clin. Pathol. 5:361.
3. Zadik P. M., Cahpman P. A. and Siddons C. A., 1993, J.Med. Microbiol., 39, 155-158.
4. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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