Technical Information

Hemorrhagic Coli (HC) Agar

Product Code: DM 2158

Application: - Hemorrhagic coli (HC) Agar is recommended for isolation and enumeration of *Escherichia coli* with an enzyme labeled monoclonal antibody.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
Sorbitol	20.000
Sodium chloride	5.000
Bile salts	1.120
Bromocresol purple	0.015
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Hemorrhagic colitis is a type of gastroenteritis in which certain strains of Escherichia coli infect the large intestine and produce a toxin that causes bloody diarrhea and other serious complications. *Escherichia coli* O157:H7 was found to be the cause of hemorrhagic colitis [1]. Outbreaks can be caused by eating undercooked beef, especially ground beef, or by drinking unpasteurized milk or juice. HC Agar is used for isolation and enumeration of *E. coli,* the main strain causing haemorrhagic colitis [2]. Casein enzymic hydrolysate in the medium is a source of carbon, nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance of the medium. *E. coli* O157:H7 doesnt ferment lactose therefore; lactose is replaced with sorbitol as fermentable carbohydrate. Bromocresol purple acts as a pH indicator. Bile salts inhibit accompanying gram-positive bacteria. Homogenize 10 grams of test sample in 90 ml Peptone Water (DM1028) (prepare 1:100 dilutions if counts are expected to be high). Pipette 1 ml aliquots through disposable 100 µm pre filter and add to 10 ml Peptone Water (DM1028) filtered through Hydrophobic Grid Membrane Filters (HGMF) [3]. Lay the filters onto Hemorrhagic Coli (HC) Agar and incubate at 43°C for 16-20 hours. Replicate colony growth onto other HGMFs using HGMF replicator. Incubate replicates on HC Agar at 43°C for 16-20 hours and test original filters with conjugated antibody [2].

Methodology

Suspend 61.13 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Quality Control

Physical Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Purple coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.11% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH Range 7.00-7.40

Cultural Response/Characteristics

DM2158: Cultural characteristics observed after an incubation at 43°C for 16-20 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Escherichia coli O157:H7	50-100	good-luxuriant	>=50%
Proteus mirabilis ATCC 25933	50-100	good	40-50%

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Riley L. W., Remis R. S., Helgerson S. D., 1983, N. Engl. J. Med., 308:681.
2. Downes F. P., and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington,. D.C.
3. Todd et al, 1988, Appl. Environ. Microbiol., 54:2536.

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