Hemorrhagic Coli (HC) Agar

M1158

Intended use

Recommended for isolation and enumeration of *Escherichia coli* with an enzyme labelled monoclonal antibody.

Composition**

Ingredients	g / L
Tryptone	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

Directions

Suspend 61.13 grams 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. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Hemorrhagic colitis is a type of gastroenteritis in which certain strains of the bacterium *Escherichia coli* infect the large intestine and produce a toxin that causes bloody diarrhea and other serious complications. *Escherichia coli* O157:H7 was recognized as 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 primary strain causing hemorrhagic colitis (2,3).

Tryptone 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 does not ferment sorbitol and produces colourless colonies. 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 (M028) (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 (M028) filtered through Hydrophobic Grid Membrane Filters (HGMF) (1). 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).

Type of specimen

Clinical samples: stool, rectal swab, etc. ; Food samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques or handling specimens as per established guidelines (4,5).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7,8). After use,contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. 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 clinical 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 tests must be carried out for complete identification.

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

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

7.00-7.40

Cultural Response

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

Store between 10-30°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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1.Riley L. W., Remis R. S., Helgerson S. D., 1983, N. Engl. J. Med., 308:681.
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3.Todd et al, 1988, Appl. Environ. Microbiol., 54:2536.
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.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
6.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc.,Washington, D.C.

7. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8. 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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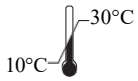
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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www.cepartner4u.eu

CE Marking

Do not use if package is damaged

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