MUG EC O157 Agar

M1373

Intended Use:

Recommended for isolation and differentiation of enterohaemorrhagic *Escherichia coli* O157:H7 from foodstuffs, water and other samples by a fluorogenic method.

Composition**

Ingredients	g / L
Casitose ▲	20.000
HM extract$	2.000
Yeast extract	1.000
Sorbitol	10.000
Ferric ammonium citrate	0.500
Sodium chloride	5.000
Bromothymol blue	0.025
Sodium thiosulphate	2.000
Sodium deoxycholate	1.120
4-Methylumbelliferyl ß-D-Glucuronide (MUG)	0.100
Agar	13.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Casein peptone
$ Equivalent to Meat extract

Directions

Suspend 54.74 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

MUG EC O157 Agar is recommended (1) for isolation and enumeration of enterohaemorrhagic *Escherichia coli* (EHEC) from foodstuffs, water and clinical samples based on sorbitol utilization and formation of beta-glucuronidase enzyme. The enterohaemorrhagic *E. coli* O157:H7 strains produce toxins, which can result in life threatening extra intestinal complications in the form of the hemolytic uremic syndrome and thrombotic-thrombocytopenic purpura. Due to severe clinical implications, the isolation and detection of *E. coli* O157:H7 strains are of importance.

Sodium deoxycholate inhibits the growth of gram-positive microbes. Sorbitol provides carbon and energy source. Bromothymol blue is the pH indicator. Microorganisms utilizing sorbitol exhibit yellow colonies whereas sorbitol-negative strains (such as *E.coli* O157:H7) grow as greenish colonies. Hydrogen suphide production is detected as black-brown colony colouration due to presence of sodium thiosulphate and ferric ammonium citrate. Thus *Proteus mirabilis* having similar biochemical characteristics as that of *E. coli* O157:H7 can easily be differentiated. 4-Methylumbelliferyl b-D-glucuronide (MUG) is converted into 4-methylumbelliferone by beta-D-glucuronidase-forming pathogens. 4-methylumbelliferone fluoresces under UV light. All commensal *E. coli* produce beta-glucuronidase. *E. coli* O157:H7 is not capable of forming b-glucuronidas, thus when exposed under long-wave UV light, no fluorescence is observed. The plates were exposed to ammonia fumes to increase fluorescence as suggested by Freir and Hartman (2).

Type of specimen

Clinical samples; Food and dairy samples; Water samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6,7). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(8) After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use. 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. Further biochemical and serological tests must be carried out for further 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 1.3% Agar gel.

Colour and Clarity of prepared medium

Bluish green coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.47% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Fluorescence (under UV)#
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	luxuriant	>=50%	yellow	negative
Escherichia coli O157:H7	50-100	luxuriant	>=50%	colourless	negative
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=50%	yellow	positive
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited	0%		
Proteus mirabilis ATCC 25933	50-100	luxuriant	>=50%	brown, may show black colouration(H2S production)	negative
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	>=50%	yellow w/black centre	negative

Key :*Corresponding WDCM numbers. # - Fluorescence can be visualized on addition of NaOH solution or exposure to ammonia fumes. (#) Formerly known as *Enterobacter aerogenes*

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 (3,4).

Please refer disclaimer Overleaf.

Reference

1. Szabo R. A., Todd E. C., Jean A., 1986, J. Food Prot., 10:768-772.
2. Freir T.A. and Hartman P.A. (1987) Appl. Env. Microbiol. 53. 1246-1250
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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.
5. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
6. 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.
7. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.
8. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

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IVD ***In vitro*** **diagnostic medical device**

30°C / 10°C **Storage temperature**

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