ECD MUG Agar

M1358

Intended Use:

Recommended for demonstrating the presence of *Escherichia coli* by fluorescence in UV and positive indole test while inhibiting accompanying intestinal flora.

Composition**

Ingredients	Gms / Litre
Tryptone	20.000
Lactose	5.000
Sodium chloride	5.000
Bile salts mixture	1.500
Dipotassium hydrogen phosphate	4.000
Potassium dihydrogen phosphate	1.500
Tryptophan	1.000
4-Methylumbelliferyl ß-D-Glucuronide (MUG)	0.070
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 53.07 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. Mx well and pour into sterile Petri plates.

Principle And Interpretation

EC Medium developed by Hajna and Perry (5) to improve the methods for the detection of coliforms and *Escherichia coli*. Feng and Hartman (3) developed a rapid assay for *E. coli* by incorporating 4-methylumbilliferyl-ß-gluconide (MUG) in to Lauryl Trytose Broth. E.C Medium with MUG is prepared according to the formula specified by the U.S Environmental Protection Agency (4) and Standard methods for water and food testing (2,9).

Tryptone provides the nitrogen, vitamins and amino acids in EC medium with MUG. Lactose is the carbon source in this medium. Bile salts mixture is the selective agent against gram-positive bacteria, particularly bacilli and fecal streptococci. Dipotassium phosphate and mono potassium phosphate are buffering agents. Sodium chloride maintains the osmotic balance of the medium. *E.coli* produces the enzyme glucoronidase that hydrolysis MUG to yield a fluorogenic product that is detectable under long wave (366 nm) UV light. Tryptophan (M1358) serves as the substrate for indole reaction.

The water sample is filtered through filter membranes, which are then placed on ECD MUG Agar and incubated overnight. After incubation observe for the presence of fluorescence under UV light. Lay a drop of Kovacs Indole reagent (R008) on the colonies. Indole positive colonies form a red zone around the colony. MUG positive and indole positive colonies are enumerated as *E. coli.*

Type of specimen

Food and dairy samples; Water samples.

Specimen Collection and Handling

For food and dairy samples follow appropriate techniques for handling specimens as per established guidelines (1,8,10). For water samples follow appropriate techniques for sample collection, processing as per guidelines and local standards (2). 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Some strains of *E. coli*, however, are MUG-negative and do not fluoresce under UV light.
2. Biochemical tests to be performed 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

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

Reaction

Reaction of 5.3% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Indole production	Fluorescence (under 366nm)
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	good-luxuriant	>=50%	negative reaction	negative
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=50%	positive reaction, red zone around the colony	positive
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	>=10^4	inhibited	0%		

Key : (*) Corresponding WDCM numbers, (#) Formerly known as *Enterbacter aerogenes*.

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 (6,7).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed.,Washington D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
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4. Federal Register 1991. National primary drinking water regulation analytical techniques, coliform bacteria. Fed Register. 56:636.
5. Hajna and Perry 1943. Am J Public Health 33:550.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. 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.
8. 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.

9. U.S Food and Drug Administration. 1995, Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
10. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.

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