

Technical Data

M1429

MUG EC O157 Agar, Modified

Intended Use:

Recommended for direct isolation and differentiation of *Escherichia coli* O157:H7 from foodstuffs and other samples.

Composition**

Ingredients	g / L
Peptone	20.000
Sodium chloride	5.000
Bile salts	1.120
Sorbitol	20.000
4-Methylumbelliferyl β-D-Glucuronide (MUG)	0.050
Bromocresol purple	0.015
Agar	12.000
Final pH (at 25°C)	7.2 ± 0.2
**Formula adjusted standardized to suit performance parameters	

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 58.18 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

Escherichia coli is one of the common organisms involved in gram-negative sepsis and endotoxin-induced shock. Enterohemorrhagic *E. coli* (EHEC) produces bloody diarrhea in humans, probably secondary to toxin damage of vascular endothelial cells (1). Patients with hemorrhagic colitis typically present abdominal cramps and watery diarrhea followed by hemorrhagic discharges resembling lower gastrointestinal tract bleeding. The enterohemorrhagic *E. coli* O157:H7 strains produce toxins, which can result in life-threatening extraintestinal complications in the form of the haemolytic uremic syndrome and thrombotic-thrombocytopenic purpura. Thus isolation and detection of *E. coli* O157:H7 strain is of public health significance.

Isolation of this serotype of E. coli is based on the fact that serotype O157:H7 is sorbitol negative.

MUG EC O157 Agar, Modified is recommended (2) for isolation and enumeration of enterohaemorrhagic *E. coli* (EHEC) from foodstuffs, water and clinical samples based on sorbitol utilization and formation of b-glucuronidase enzyme. Bile salts inhibit the growth of gram-positive microbes. Sorbitol provides carbon and energy source. Bromocresol purple is pH indicator. Microorganisms utilizing sorbitol exhibit yellow colonies whereas sorbitol-negative strains (such as *E.coli* O157:H7) grow as colourless colonies. MUG is cleaved by b-glucuronidase forming pathogens and can be detected by fluorescence under UV light. The plates are exposed to ammonia fumes to increase fluorescence as suggested by Freir and Hartman (3). All commensal *E. coli* produce b-glucuronidase and therefore cleave MUG and appear fluorescent when observed under long wave UV light (366 nm). *E. coli* O157:H7 is not capable of forming b-glucuronidase, thus when exposed to long-wave UV light, no fluorescence is observed.

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 (4,5). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7,8). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(9) 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 1.2% Agar gel.

Colour and Clarity of prepared medium

Light purple coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.8% w/v aqueous solution at 25°C. pH : 7.2±0.2

pН

7.00-7.40

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 16-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Sorbitol	Fluorescence (Under UV) [#]
<i>Bacillus cereus</i> ATCC 10876	50-100	none to poor	<=10%			
** Bacillus spizizenii ATCC 6633 (00003*)	>=10 ⁴	inhibited	0%			
Escherichia coli O157:H7	50-100	luxuriant	>=50%	colourless	negative reaction	negative
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=50%	yellow	positive reaction	positive
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	>=10 ⁴	inhibited	0%			
Serratia marcescens ATCC 8100	50-100	luxuriant	>=50%	pink	positive reaction	negative

Key : *Corresponding WDCM numbers. # - Fluorescence can be visualized by addition of NaOH solution or exposure to ammonia fumes. **Formerly known as *Bacillus subtilis* subsp. *spizizenii*

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 (4,5).

Reference

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