



MUG Lauryl Sulphate Broth, Modified

M1046I

Intended Use

Recommended as a selective medium for enumeration of presumptive *Escherichia coli* and other coliforms from milk and milk products. The composition and performance criteria of this medium are as per the specifications laid down in ISO 11866-2:2005.

Composition**

ISO 11866-2:2005 Specification-MUG Lauryl Sulphate Broth, Modified

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Ingredients	g / L	Ingredients	g / L
Tryptose	20.000	Tryptone	20.000
Lactose	5.000	Lactose	5.000
Sodium chloride	5.000	Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.750	Dipotassium hydrogen phosphate	2.750
Potassium dihydrogen phosphate	2.750	Potassium dihydrogen phosphate	2.750
Sodium lauryl sulphate	0.100	Sodium lauryl sulphate	0.100
4-Methylumbelliferyl β -D-glucuronide (MUG)	0.100	4-Methylumbelliferyl β -D-glucuronide (MUG)	0.100
Tryptophan	1.000	Tryptophan	1.000
Final pH (at 25°C)	6.8±0.2	Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36.7 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes with inverted Durham's tubes as required, taking into account the volume of the samples to be tested. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

The coliform group consists of several genera of bacteria belonging to the family *Enterobacteriaceae*. Lauryl Sulphate Broth formulated by Mallmann and Darby (1) is recommended by APHA for the detection and enumeration of coliform organisms in foods, water and waste water (2, 3). MUG is added in Lauryl Sulphate Broth as the fluorogenic compound which permits the rapid detection of *Escherichia coli* when observed under UV light where further confirmation is not required (2, 4). MUG Lauryl Sulphate Broth, Modified is recommended by the ISO Committee (5) for enumeration of presumptive *E.coli* and other coliforms from milk and milk products.

E.coli possesses the enzyme beta-glucuronidase and is capable of cleaving the fluorogenic substrate 4-Methylumbelliferyl β -D-glucuronide (MUG) with the corresponding release of the fluorogen.

MUG detects anaerogenic strains, which may not be detected in the conventional procedure. Feng and Hartman (6) used MUG-containing medium for studying β -glucuronidase activity and found that *E.coli* has 96-100% activity, while *Salmonella* species has 17% and *Shigella* species 40% activity and other genera were negative. For weakly positive strains, incubation should be carried out overnight. Robison (4) achieved no false negative results and about 5% false positive results. Tryptone provides nutrients while lactose acts as energy source. Sodium lauryl sulphate inhibits many organisms other than coliforms. 4-methylumbelliferyl- β -D-glucuronide is hydrolyzed by the enzyme β -glucuronidase to yield 4-methylumbelliferone, a fluorescent end product.

Inoculate 10 ml of the test specimen into three tubes each of single strength and double strength. Incubate the tubes at 35°C for 24 hours. Observe for opacity and gas formation. For confirmation of presumptive *E.coli*, observe for fluorescence and perform indole reaction using Kovacs Reagent (R008) (6).

Type of specimen

Dairy samples: Milk and milk products

Specimen Collection and Handling:

For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2, 5, 7,8). 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 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.

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

Colour and Clarity of prepared medium

Light amber coloured clear solution without any precipitate

Reaction

Reaction of 3.67% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 30°C for 24 hours ± 2 h. If, at this stage, neither gas formation nor opacity preventing the observation of gas formation is observed, incubate for up to 48 h ± 2 h.

Organism	Inoculum (CFU)	Growth	! Fluorescence under uv at 366nm	Indole production
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	positive	positive reaction, red ring at the interface of the medium
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50-100	luxuriant	negative	negative reaction

Key : * Corresponding WDCM numbers, # Formerly known as *Enterobacter aerogenes*, ! On addition of 0,5 ml of the sodium hydroxide solution

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. Use before expiry date on the label. 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 (9,10).

Reference

1. Mallmann and Darby, 1941, Am.J. Public Health, 31:127.
2. 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.
3. Lipps WC, Braun-Howland EB, Baxter TE, eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
4. Robison, 1984, Appl. Environ. Microbiol., 48:285.
5. International Organization for Standardization (ISO) Milk and milk products -- Enumeration of presumptive *Escherichia coli* -- Part 2: Most probable number technique using 4-methylumbelliferyl-beta-D-glucuronide (MUG), ISO 11866-2-1997
6. Feng P.C.S. and Hartman P. A., 1982, Appl. Environ. Microbiol., 43:1320.
7. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
9. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
10. 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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