LAURYL SULPHATE BROTH MUG IDF FORMULATION

Dehydrated culture medium

1 - INTENDED USE

Selective, fluorogenic medium for the enumeration of *Escherichia coli* and coliforms in milk, dairy products and other materials of sanitary importance.

2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose	20.00 g
Lactose	5.00 g
Sodium chloride	5.00 g
Sodium lauryl sulphate	0.10 g
Dipotassium hydrogen phosphate	2.75 g
Potassium dihydrogen phosphate	2.75 g
L-tryptophan	1.00 g
4-methylumbelliferone beta-D-glucuronide (MUG)	0.10 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lauryl Sulfate Broth (LSB) was first introduced by Mollan and Ourby.[1] Rapid assay for *E. coli* was developed by Feng and Harman[2] by using LSB supplemented with the compound 4-methylumbelliferone glucuronide (MUG), which is hydrolysed by glucuronidase to yield a fluorogenic product.
Lauryl Sulphate Broth with MUG (LSB MUG) is recommended by ISO 11866-1:2005 [IDF 170-1:2005][3] for simultaneous detection of *E. coli* and coliforms in milk and dairy products and by FDA-BAM[4] and AOAC[5] for detecting *E. coli* in chilled or frozen foods, exclusive of shellfish. The broth is specifically designed to allow rapid multiplication and copious gas production from a small inoculum of target organisms.[6]
Essential growth factors are provided by tryptose which is a source of nitrogen, carbon, amino acids and minerals; lactose is a fermentable carbohydrate. Phosphates act as buffer system and sodium chloride maintains the osmotic balance. The surface-active agent sodium lauryl sulphate acts as the selective agent in restricting the growth of bacteria other than coliforms.[6] Coliforms grown in LSB MUG, ferment lactose and produce gas while other bacteria are either inhibited or grow without producing gas. MUG is cleaved by β-D-glucuronidase produced by *E. coli* to 4-methylumbelliferone and glucuronide; the fluorogenic 4-methylumbelliferone can be determined directly by using a long-wave ultraviolet light (Wood's lamp). The presence of 1 g/L tryptophan enhances the detection of tryptophanase by the indole reaction.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 36.7 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute 10 mL into 16 x160 mm test tubes containing inverted Durham tube. Sterilise by autoclaving at 121°C for 15 minutes. In the case of double strength suspend 71.2 g in 1000 ml of cold purified water and dispense 10 mL into 20x200 mm tubes. The Durham tubes shall not contain air bubbles after sterilization.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared tubes appearance	light yellow, limpid
Final pH at 20-25 °C	6.8 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Lauryl Sulfate Broth MUG IDF Formulation	Dehydrated medium	401580F2	500 g (13.6 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, test-tubes, Durham tubes, Wood's lamp, ancillary culture media and reagents.

8 – SPECIMENS

Milk, dairy products, chilled or frozen foods, exclusive of shellfish. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

Take 3 tubes of double strength and 3 tubes of single-strength medium. Inoculate to each tube 10 mL and 1 mL respectively of the test sample if liquid or of the initial suspension. Proceed in the same way for each dilution.
Carefully mix the inoculum with the medium.
Incubate the tubes at 30 °C ± 1 °C for 24 ± 2 hours. If, at this stage, neither gas formation nor opacity preventing of gas formation is observed, incubate for up to 48 ± 2 hours.

10 - READING AND INTERPRETATION

Presence of growth and gas production are considered a positive test for the presence of coliforms.
Perform the confirmatory test for *E coli* on all tubes:

- add to each tube 0.5 ml of NaOH 0.1M and observe for fluorescence under a Woods lamp
- add to each tube showing fluorescence 0.5 ml of Kovacs Reagent (cat. n° 19171000). Mix well and examine after 1 minute for the formation of a distinct red-purple colour in upper layer.

Identify those tubes which develop fluorescence and are positive to indole test as *E. coli.*

11 - USER QUALITY CONTROL

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All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	30°C/24H/A	growth, with fluorescence under Wood's lamp and gas
C. freundii ATCC 43864	30°C/24H/A	growth with gas, without fluorescence under Wood's lamp
E. faecalis ATCC 19433	30°C/48 H/A	partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 – PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Lauryl Sulfate Broth MUG IDF Formulation is tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 30°C for 24 and 48 hours and recording the highest dilution showing growth, gas production, fluorescence under Wood's lamp, positivity to direct indole test, in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}).
Productivity is tested with the following target strains: *E. coli* ATCC 25925 and *E. coli* ATCC 8739. The productivity index Gr_{RB}-Gr_{TB} for each test strain is ≤ 1 and the tubes exhibit gas into the Durham tubes, fluorescence under Wood's lamp and positivity to indole test.
Specificity is tested with appropriate dilutions of coliforms strains *C.freundii* ATCC 43864 and *K.pneumoniae* ATCC 27736 and the lactose negative strain S. Typhimurium ATCC 14028. After incubation, the coliform strains exhibit good growth with gas, without fluorescence under Wood's lamp and negative indole test whereas *Salmonella* strain exhibit good growth without gas, without fluorescence under Wood's lamp and negative indole test.
Selectivity is tested with appropriate dilutions of non-target strain *E.faecalis* ATCC 19433. After incubation, the growth of non-target strain is partially inhibited.

13 - LIMITATIONS OF THE METHOD

- It has been reported that approximately 40% of *Shigella* species, various bio-serotypes of *Salmonella* (13% of *Salmonella* subgenus I) may be β-glucuronidase positive and fluorescent under Wood's lamp; only exceptionally this test is positive with *Providencia*, *Enterobacter* and *Yersinia* strains (1-5%).[7-9]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains.[8]
- Up to 10% of *E. coli* have been reported to be slow or non-lactose fermenting but should be MUG-positive.[4,10]

14 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The ante and post mortem controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +2°C / +8°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging).

16 - REFERENCES

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3. ISO 11866-1:2005 [IDF 170-1:2005] Milk and milk products — Enumeration of presumptive Escherichia coli — Part 1: Most probable number technique using 4-methylumbelliferyl-beta-D-glucuronide (MUG).
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5. Moberg, L.J., M.K. Wagner, and L.A. Kellen. Fluorogenic assay for rapid detection of Escherichia coli in chilled and frozen foods: collaborative study. J. Assoc.Off. Anal. Chem.1988; 71:589-602.
6. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 195-196.

7. Trepeta RW, Edberg SC. Methylumbelliferyl- D-glucuronide-based medium for rapid isolation and identification of E. coli. J Clin Microbiol 1984; 19 :172.'
8. Robison, B.J. 1984. Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl. Environ. Microbiol. 48:285-288
9. Kaluzewski SD, Tomczuk D. Evaluation of the Usefulness of Tests for Productionof Beta-D-glucuronidase and Propylene Glycol Utilization for the Differentiation of Enterobacteriaceae Rods. Med Dosw Mikrobiol, 1995; 47:155-68.
10. Gokul Yaratha, MD, Sarah Perloff, DO, Kinesh Changala, MBBS. Lactose vs non-lactose fermenting E. coli: Epidemiology, Clinical Outcomes, and Resistance. Open Forum Infect Dis 2017; V4 (Suppl 1)

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2023/02

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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