

LACTOSE BROTH

Dehydrated and ready-to-use culture medium

1 - INTENDED USE

Non-selective medium for detection and enumeration of coliform bacteria in materials of sanitary importance.

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

Beef extract	3 g
Gelatin peptone	5 g
Lactose	5 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

Lactose broth was formulated by American Public Health Association (APHA) to test dairy products and water^{1,2} and it is no longer included in recent editions. Lactose Broth may be used for study of lactose fermentation of bacteria in general and it is reported by FDA-BAM and as an alternative to Lauryl Sulfate Broth in the presumptive MPN test for total coliforms and faecal coliforms in foodstuffs³ and as a pre-enrichment medium for detection of *Salmonella* in some foods⁴. The medium is indicated by ISTISAN Report 96/35 for the enumeration of *E. coli* in frozen pasta using the MPN technique.⁵ Essential growth factors are provided by beef extract and gelatin peptone which are sources of nitrogen, carbon, amino acids and minerals. Lactose is the fermentable carbohydrate for coliforms.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 13 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute 10 mL into test tubes containing inverted Durham tube or 225 mL into flasks. Sterilise by autoclaving at 121°C for 15 minutes. In the case of double strength, suspend 26 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	very pale yellow, limpid
Final pH at 20-25 °C	6.9 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Lactose Broth	Dehydrated medium	4015752	500 g (38.5 L)
Lactose Broth	Ready-to-use tubes	551575	20 x 10 mL

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, ancillary culture media and reagents.

8 - SPECIMENS

Foods and materials of sanitary importance. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.^{3,4}

9 - TEST PROCEDURE

Coliforms and faecal coliforms (MPN technique)³

- Prepare decimal dilution of the sample with the suitable diluent.
- Using at least 3 consecutive dilutions, inoculate 1 mL aliquots from each dilution into 3 Lactose Broth tubes for a 3 tube MPN analysis (some analysis may require the use of 5 tubes for each dilution).
- Incubate Lactose Broth tubes at 35°C ± 0.5°C. Examine tubes and record reactions at 24 ± 2 h for gas.
- Re-incubate gas-negative tubes for an additional 24 h and examine and record reactions again at 48 ± 3 h.
- Perform confirmed test on all presumptive positive (gas) tubes.
- Completed test for coliforms: from each gassing lactose broth tube, transfer a loopful of suspension to a tube of Brilliant Green Bile Broth (REF 401265) and incubate at 35°C ± 0.5°C and examine for gas production at 48 ± 3 h.
- Completed test for faecal coliforms: from each gassing Lactose Broth tube transfer a loopful to a tube of EC broth (REF 401425) and incubate 24 ± 2 h at 44.5°C and examine for gas production. If negative, re incubate and examine again at 48 ± 2 h.

10 - READING AND INTERPRETATION

The medium becomes turbid when bacteria are growing; gas formation can be observed as bubbles production accumulated into Durham tubes.

Consider as coliforms the growth in Lactose Broth that show gas formation in the Durham tube of Brilliant Green Bile Broth test tubes incubated at 35°C for 24-48 hours.

Consider as faecal coliforms the growth in Lactose Broth that show gas formation in the Durham tube of EC Broth test tubes incubated at 44.5°C for 24-48 hours.

11 - USER QUALITY CONTROL

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
<i>E. coli</i> ATCC 25922	35° C/24 H/A	growth, with gas production
<i>C. freundii</i> ATCC 43864	35° C/24 H/A	growth, with gas production

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 and ready to use Lactose Broth are tested for productivity and specificity 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 35°C for 24 hours and recording the highest dilution showing growth and gas production in Reference Batch (G_{RB}) and in Test Batch (G_{TB}). Productivity is tested with the following target strains: *E. coli* ATCC 8739, *C. freundii* ATCC 43869, *E. aerogenes* ATCC 13048, *K. pneumoniae* ATCC 27736. The productivity index $G_{RB}-G_{TB}$ for each test strain is ≤ 1 and the tubes exhibit gas into the Durham tubes. Specificity is tested with appropriate dilutions of non-target strain *S. Enteritidis* ATCC 13076. After incubation, the strain exhibits good growth without gas production.

13 - PRECAUTIONS AND WARNINGS

- This culture medium 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 this 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.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Ready-to-use tubes are subject to terminal sterilization by autoclaving
- Each ready-to-use tube of this culture medium is for single use only.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium 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 Sheets of the products 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

Upon receipt, store at +10°C /+30°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). According to MacFaddin the self-prepared screwcap tubes and flasks may be stored at +2°C /+8°C for 6 months.⁶

Ready-to-use medium in tubes

Upon receipt, store tubes in their original pack at +2°C /+8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. Tubes from opened secondary packages can be used up to the expiration date. Opened tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).














15 - REFERENCES

1. American Public Health Association. Standard methods for the examination of dairy products, 1948. 9th ed. APHA, New York, N.Y.
2. American Public Health Association. Standard methods for the examination of water and sewage, 9th ed. 1946. APHA, New York, N.Y.
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria. Rev October 2020.
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev March 2022.
5. Rapporto ISTISAN 96/35. ISSN 1123-3117. Metodi di analisi per il controllo microbiologico degli alimenti. Raccolta a cura di D.De Medici, L.Feniccia, L.Orefice e A.Stacchini
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.





TABLE OF APPLICABLE SYMBOLS

 or  Catalogue number	 Batch code	 Manufacturer	 This side up	 Store in a dry place	 Fragile
 Temperature limitation	 Content sufficient for <n> tests	 Consult Instructions for Use	 Use by	 Keep away from direct light	 For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/08

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

