FERMENTATION BROTH BASE
FERMENTATION BROTH RHAMNOSE
FERMENTATION BROTH XYLOSE

Dehydrated and ready-to-use culture medium

1 - INTENDED USE

Supplemented with carbohydrates, Fermentation Broth Base is used for the determination of fermentation reactions of microorganisms.

2 - COMPOSITION - TYPICAL FORMULA *

DEHYDRATED FERMENTATION BROTH BASE (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	10.0 g
Beef extract	1.0 g
Sodium chloride	5.0 g
Bromocresol purple	0.02 g

FERMENTATION BROTH RHAMNOSE (READY-TO USE TUBES)

Peptone	10.0 g
Beef extract	1.0 g
Sodium chloride	5.0 g
Bromocresol purple	0.02 g
L-rhamnose	5.00 g
Purified water	1000 mL

FERMENTATION BROTH XYLOSE (READY-TO USE TUBES)

Peptone	10.0 g
Beef extract	1.0 g
Sodium chloride	5.0 g
Bromocresol purple	0.02 g
D-xylose	5.00 g
Purified water	1000 mL

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Carbohydrate fermentation tests detect the ability of microorganisms to ferment a specific carbohydrate. Fermentation patterns can be used to differentiate among bacterial groups or species.
Fermentation Broth Base is formulated as recommended by ISO 11290[1,2] (Carbohydrate Utilization Broth) and by FDA-BAM[3] (Purple Carbohydrate Fermentation Broth Base).
ISO 11290 recommends the medium supplemented with L-rhamnose and D-xylose in the confirmation procedure of *Listeria monocytogenes*.
FDA-BAM recommends the medium supplemented with dulcitol, lactose, sucrose in the confirmation procedure of *Salmonella*[4] and supplemented with dextrose, esculin, maltose, rhamnose, mannitol, and xylose in the confirmation procedure of *Listeria monocytogenes*[5].
The basal medium contains a peptone with a low carbohydrates content and beef extract which are sources of nitrogen, carbon and minerals for bacterial growth. Sodium chloride maintains the osmotic balance. Bromocresol purple is a pH indicator: when Fermentation Broth Base is prepared with a supplemented carbohydrate, most of the end products of its fermentation are organic acids, which produce a colour change of the pH indicator from purple to yellow; if the test is negative, a catabolic attack of peptones will occur with the formation of ammonia, the alkalinisation of the medium and a colour change of indicator to darker purple. A Durham tube can be inserted into the test tube to record gas production: if gas is produced during the fermentation reaction, it is collected in the inverted Durham tube.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 16 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Dispense the medium into tubes of suitable capacity to obtain portions appropriate for the test. Sterilize for 15 min in the autoclave at 121 °C.
Prepare a filter sterilized 5% solution of the suitable carbohydrate (e.g., 5 g of L-rhamnose or D-xylose in 100 mL of purified water).
For each carbohydrate, add aseptically x mL of carbohydrate solution to 9x mL of the Fermentation Broth Base (e.g. 2.7 mL of Fermentation Broth Base + 0.3 mL of carbohydrate solution or 4.5 mL of Fermentation Broth Base + 0.5 mL of carbohydrate solution).
Dispense the medium with Durham fermentation tubes, if gas formation is to be recorded.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tube appearance	pale violet, limpid
Final pH at 20-25 °C	6.8 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Fermentation Broth Base	Dehydrated medium	4014882	500 g (31.2 L)
Fermentation Broth Rhamnose	Ready-to-use tubes	521488R	24 x 3 mL
Fermentation Broth Xylose	Ready-to-use tubes	521488X	24 x 3 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Erlenmeyer flasks, tubes, Durham fermentation tubes, ancillary culture media and reagents, carbohydrates (e.g., rhamnose and xylose).

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

8 – SPECIMENS
Pure culture of bacterial strains under examination.

9 - TEST PROCEDURE
Using a loop, aseptically inoculate each tube with a pure culture of the strain under examination obtained from a non-selective agar (e.g Tryptic Soy Yeast Extract Agar REF 402166).
Swirl the tube gently to mix contents and incubate at 37°C for 24 h to 48 h.
Prolonged incubation may be required to be considered a negative result.[6]
Inoculate and incubate also a tube without the addition of carbohydrates (control tube).

10 - READING AND INTERPRETATION
Positive reactions (acid formation) are indicated by a yellow colour which occur mostly within 24 h to 48 h for microvolumes tubes, and up to 5 days for macro volumes tubes.
Positive reaction (carbohydrate degradation): the medium is turbid, turns yellow and the formation of gas bubbles can be observed, if Durham tubes are present.
Negative reaction: the medium is turbid and remains purple or changes to deep purple.
No yellow colour should appear in the control tube.
After a positive reaction has been observed, discard the tube; by prolonging the incubation, an inversion of the reaction may be observed.

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 the test strains useful for the quality control of the medium supplemented with rhamnose.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
L. monocytogenes ATCC 13932	37°/ 24-48 H / A	the medium turns to yellow
L. ivanovii ATCC 19119	37°/ 24-48 H / A	the medium doesn't turn to yellow

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

12 – PERFORMANCES CHARACTERISTICS
Prior to release for sale, representative samples of all lots of dehydrated Fermentation Broth Base supplemented with xylose and rhamnose and ready-to-use tubes are tested for specific colour change of the tubes by comparing the results with a previously approved Reference Batch.
The medium is tested by inoculation the tubes with pure culture of *L. monocytogenes* ATCC 13932, *L. monocytogenes* NCTC 7973 and *L. ivanovii* ATCC 19119. After incubation at 37°C for 24-48 hours the strains exhibit the following reactions:
Fermentation Broth Base + rhamnose: *L. monocytogenes* turns the medium to yellow, *L. ivanovii* doesn't turn the medium to yellow
Fermentation Broth Base + xylose: *L. monocytogenes* doesn't turn the medium to yellow, *L. ivanovii* turns the medium to yellow

13 – LIMITATIONS OF THE METHOD
- There exist rare strains of *L. monocytogenes* which do not ferment L-rhamnose.[1]
- Carbohydrate fermentation is one of the tests used to identify pure bacterial cultures. For complete identification, other suitable tests must be carried out.

14 - 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 Material Safety Data Sheets.
- 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.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Each 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 Sheet 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

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).

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).

16 – REFERENCES

1. ISO 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1: Detection method
2. ISO 11290-2:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2: Enumeration method.
3. U.S. Department of Health and Human Services, F.D.A. Bacteriological Analytical Manual, M130.
4. U.S. Department of Health and Human Services, F.D.A. Bacteriological Analytical Manual, Chapter 5: Salmonella, March 2022.
5. U.S. Department of Health and Human Services, F.D.A. Bacteriological Analytical Manual, Chapter 10: Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods, April 2022.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT 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	2023/01

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

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