

# ROGOSA BIOS BROTH

## Dehydrated culture medium

### 1 - INTENDED USE

Culture medium for the selective isolation and enumeration of lactobacilli.

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

Peptozimatic	2.00 g
Tryptone	4.00 g
Yeast extract	9.00 g
Glucose	10.00 g
Arabinose	5.00 g
Sucrose	5.00 g
Sodium acetate	15.00 g
Ammonium citrate	2.00 g
Potassium dihydrogen phosphate	6.00 g
Magnesium sulphate	0.57 g
Manganous sulphate	0.12 g
Ferrous sulphate	0.03 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

Lactobacilli are large, Gram-positive aerotolerant anaerobes or microaerophilic, rod-shaped, non-spore-forming bacteria. Lactobacilli are regarded as beneficial members of the human microbiota at a number of body sites, such as oral cavity, gastro-intestinal tract, and female genital system, but they can infrequently act as opportunistic pathogens in both children and adults.<sup>1</sup>

Lactobacilli are particularly associated with advanced dental caries where they are considered a secondary colonizer but probably play a role in exacerbating existing lesions and have been associated to a multitude of various infections including bacteriemia, endocarditis, peritonitis, chorioamnionitis, meningitis and intra-abdominal abscesses.<sup>1</sup> The depletion of lactobacilli from the vaginal microbiota and the increased bacterial diversity are characteristic feature of bacterial vaginosis.<sup>1</sup>

Rogosa Bios Broth is prepared according to a modification of the formula proposed by Rogosa, Mitchell and Wiseman<sup>2,3</sup> and is intended for the isolation and enumeration of lactobacilli.<sup>1,4,5</sup>

The medium contains two peptones and yeast extract as sources of nitrogen, carbon and vitamins, necessary for microbial growth. Dextrose, arabinose and sucrose provide carbon and are sources of energy. Tween 80 acts as surfactant and provides fatty acids required for the metabolism of lactobacilli. Ammonium citrate and sodium acetate inhibit the growth of streptococci, moulds, and other oral microbial flora and restrict *Proteus* swarming. Potassium dihydrogen phosphate buffers the medium. Magnesium sulphate, ferrous sulphate and manganous sulphate are sources of inorganic ions for the optimal growth of lactobacilli. Acetic acid reduces the pH of the medium to acidic values.

### 4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 58.7g in 1000 mL of cold purified water. Add 1 mL of Tween 80 and 1.32 mL of 96% glacial acetic acid. Heat to boiling with frequent agitation, boil for 2-3 minutes. Do not autoclave.

### 5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	Yellowish, fine, free-flowing powder
Solution and plated medium appearance	yellow, limpid
Final pH at 20-25°C	5.4 ± 0.2

### 6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Rogosa Bios Broth	Dehydrated medium	4019902	500 g (8.5 L)

### 7 - MATERIALS REQUIRED BUT NOT PROVIDED

Water-bath, incubator and laboratory equipment as required, Erlenmeyer flasks, Petri dishes, sterile loops and swabs, controlled atmosphere generators and jars, ancillary culture media and reagents for the identification of the colonies.

### 8 - SPECIMENS

Rogosa Bios Broth tubes can be directly inoculated with a variety of specimens. Good laboratory practices for collection, transport and storage of the specimens should be applied. The medium is not suitable for isolation of dairy lactobacilli.<sup>4</sup>

### 9- TEST PROCEDURE

Inoculate the specimen directly into test tubes.

Incubate for 3 days at 35°C or for 5 days at 30°C.<sup>2</sup> Lactobacilli prefer a microaerophilic atmosphere, therefore an incubation in a 5-10% CO<sub>2</sub>-supplemented atmosphere or in anaerobic conditions are recommended by some authors.<sup>2,3,4</sup>

### 10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth (turbidity of the medium in tubes).

### 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, it is responsibility of the end-user to perform Quality Control testing 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>L.rhamnosus</i> ATCC 7469	35-37°C / 44-48 H	growth
<i>S.aureus</i> ATCC 25923	35-37°C / 44-48 H	inhibited

CO<sub>2</sub>: 5-10% CO<sub>2</sub>; 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 Rogosa Bios Broth REF 401990 is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by dilution to extinction technique with suitable dilutions of the the following target strains: *L.casei* ATCC 7469, *L.sake* ATCC 15521, *L.fermentum* ATCC 9338, wild strains of *L.bulgaricus*, *L.acidophilus*, *L.mesenteroides*. After incubation at 35-37°C for 44-48 hours all target strains exhibit a good growth.

Selectivity is evaluated by inoculating the tubes with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target organisms *E.coli* ATCC 25922, *E.faecalis* ATCC 29212, and *C.albicans* ATCC 10231. After incubation at 35-37°C for 44-48 hours the growth of non-target strains is partially inhibited.

### 13 - LIMITATIONS OF THE METHOD

- In general *Lactobacillus* spp. can be selectively cultured using media with an acidic pH such as Rogosa medium, though some more fastidious strains may not grow on these media.<sup>1</sup>
- It is advisable to inoculate, together with Rogosa Bios Broth, conventional blood agar media.<sup>1</sup>
- The medium should not be used for maintenance of lactobacilli; transfer colonies for further tests as soon as possible.<sup>4</sup>
- The salt in the formulation makes the medium unsuitable for isolation of dairy lactobacilli: *L.lactis*, *L.bulgaricus* and *L.helveticus*.<sup>4</sup>
- Other organisms such as enterococci, pediococci and *Leuconostoc* species may grow on this medium.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

### 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 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](http://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](http://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 for the validation of the shelf life of the finished products, according to the type (tubes/bottles) and the storage method (temperature and packaging).











### 16 - REFERENCES

1. Butler-Wu SM, She RC. *Actinomyces*, *Lactobacillus*, *Cutibacterium* and other non-spore-forming Gram-positive rods. In Carrol KC, Pfaller MA *et al.* editors. Manual of clinical microbiology, 12th ed. Washington, DC: American Society for Microbiology; 2019.
2. Rogosa M, Mitchell JA, Wiseman RF. A selective medium for the isolation and enumeration of oral and fecal lactobacilli J Bact 1951; 62:132
3. Rogosa M, Mitchell JA, Wiseman RF. A selective medium for the isolation and enumeration of oral lactobacilli. J Dent Res 1951; 30(5):682
4. 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	 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	2022/05

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

