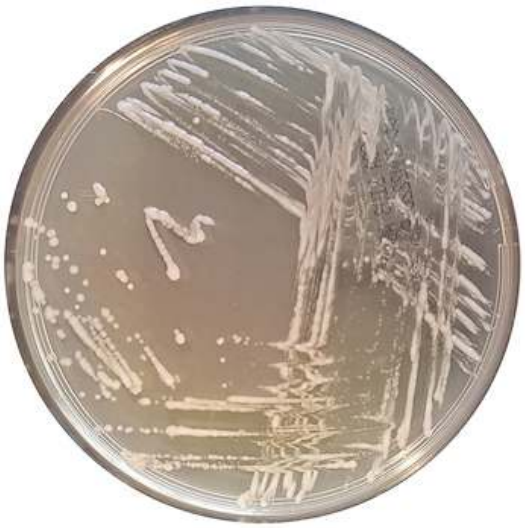
INSTRUCTIONS FOR USE

ROGOSA BIOS AGAR

Dehydrated culture medium



L.rhamnosus on Rogosa Bios Agar

1 - INTENDED USE

In vitro diagnostic. Culture medium for the selective isolation and enumeration of lactobacilli from clinical specimens and foodstuffs.

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
Agar	15.00 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.[1]
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.[1] The depletion of lactobacilli from the vaginal microbiota and the increased bacterial diversity are characteristic feature of bacterial vaginosis.[1]
Rogosa Bios Agar is prepared according to a modification of the formula proposed by Rogosa, Mitchell and Wiseman[2,3] and is intended for the isolation and enumeration of lactobacilli from clinical specimens and foodstuffs.[1,4,5]
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 73.7 g 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. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

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 Agar	Dehydrated medium	4019852	500 g (6,8 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 Agar plates can be directly inoculated with a variety of clinical specimens such as faeces, saliva, vaginal specimens.[4,5] Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[1] Collect specimens before antimicrobial therapy where possible. Rogosa Bios Agar may be used for inoculation of foodstuffs: consult appropriate Standard Methods for detailed information.[6] The medium is not suitable for isolation of dairy lactobacilli.[4]

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

9- TEST PROCEDURE

Allow plates to come to room temperature. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. For quantitative evaluation use appropriate inoculation techniques.
Incubate for 3 days at 35°C or for 5 days at 30°C.[2] Lactobacilli prefer a microaerophilic atmosphere, therefore an incubation in a 5-10% CO_2-supplemented atmosphere or in anaerobic conditions are recommended by some authors.[2,3,4]

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Lactobacilli appear as large (2-3 mm in diameter), whitish, smooth, circular colonies.
Other lactic acid bacteria may also grow on this medium and produce similar types of colonies. Most other organisms are inhibited on Rogosa Bios Agar although enterococci and pediococci may show delayed growth. Both enterococci and pediococci will produce very small colonies with a diameter of 0.5 to 1.0 mm.

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
L.rhamnosus ATCC 7469	35-37°C / 44-48 H / CO_2	growth
S.aureus ATCC 25923	35-37°C / 44-48 H / CO_2	inhibited

CO_2: 5-10% CO_2; 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 Agar REF 401985 is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by quantitative method and by semi-quantitative ecometric technique with the following target strains: *L.plantarum* ATCC 8014, *L.rhamnosus* ATCC 7469, *L.gasseri* ATCC 19992, *L.acidophilus* ATCC 314. After incubation at 35-37°C for 44-48 hours in a 5-10% CO_2-supplemented atmosphere all target strains show a good growth with whit whitish colonies.

Selectivity is evaluated by modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target organisms *S.lactis* ATCC 11454, *E.coli* ATCC 25922, *E.faecalis* ATCC 29212, and *C.albicans* ATCC 10231. After incubation at 35-37°C for 44-48 hours in a 5-10% CO_2-supplemented atmosphere, the growth of *S.lactis*, *E.faecalis* and *E.coli* is inhibited while *C.albicans* is partially inhibited.

13 - LIMITATIONS OF THE METHOD

- In general *Lactobacillus* spp. can be selectively cultured using agar media with an acidic pH such as Rogosa medium, though some more fastidious strains may not grow on these media.[1]
- It is advisable to inoculate, together with Rogosa Bios Agar, conventional blood agar media.[1]
- The medium should not be used for maintenance of lactobacilli; transfer colonies for further tests as soon as possible.[4]
- The salt in the formulation makes the medium unsuitable for isolation of dairy lactobacilli: *L.lactis, L.bulgaricus* and *L.helveticus*.[4]
- Other organisms such as enterococci, pediococci and *Leuconostoc* species may grow on this medium.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If required and relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious disease; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of the microscopic and/or other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, 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.
- Apply Good Manufacturing Practice in the production process of prepared media.
- 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.
- 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 media 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.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- 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 (plates/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.
5. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition. CRC Press,1997.
6. Hall, Ledenbach and Flowers. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C. 2001.

TABLE OF APPLICABLE SYMBOLS

REF Or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/03
Revision 2	Removal of obsolete classification	2023/04

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

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