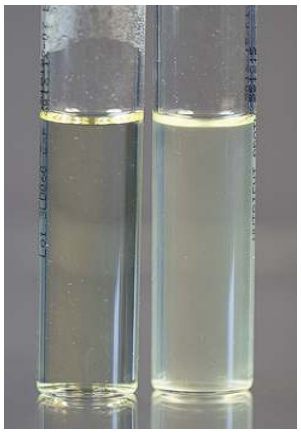
INSTRUCTIONS FOR USE

NUTRIENT BROTH

Dehydrated culture medium



Nutrient Broth From left: un-inoculated tube, growth of *E.faecalis*

1 - INTENDED USE

In vitro diagnostic. General purpose liquid medium for the cultivation of non-fastidious microorganisms isolated from clinical and non-clinical specimens.

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

Beef extract	3 g
Peptone	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

Nutrient Broth is a medium suitable for the cultivation of non-fastidious microorganisms isolated from clinical and non-clinical specimens. It was included in several Standards for the examination of water, food, dairy products[1-3]; it is not a recommended bacteriological medium in later editions of these publications.
Nutrient Broth is one of several non-selective media useful in routine cultivation of microorganisms. It can be used for the sub-culture of colonies grown on other media to be tested with bacteriological and serological assays.
The peptone and meat extract provide carbon, nitrogen, vitamins and minerals sufficient for the growth of most non-fastidious microorganisms (*Enterobacteriaceae*, enterococci, staphylococci, etc.).

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 8 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute and sterilize by autoclaving at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	light brown, fine, homogeneous, free-flowing powder
Solution appearance	very light yellow, limpid
Final pH at 20-25 °C	6.8 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Nutrient Broth	Dehydrated medium	4018152 4018154	500 g (62.5 L) 5 kg (625 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops, needles and swabs, incubator and laboratory equipment as required, microbiological tubes and flasks, Erlenmeyer flasks, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Nutrient Broth can be used for the sub-culture of colonies grown on primary isolation media. It is not suitable for the direct inoculation of clinical specimens.

9 - TEST PROCEDURE

With a bacteriological needle or loop inoculate the liquid medium in the test tube with a colony grown on a plating medium.
Routinely, incubate at 35-37°C in aerobic conditions for 18-24 hours.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

10 - READING AND INTERPRETATION

The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of growth is closely related to the type or types of microorganisms grown.

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
E. faecalis	ATCC 19433	35-37°C / 18-24H / A	good growth
E. coli	ATCC 25922	35-37°C / 18-24H / A	good growth

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

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12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Nutrient Broth (Test Batch: TB), is tested for productivity by comparing the results with a previously approved Reference Batch (RB).
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of organisms in test tubes and incubating at 35-37° for 18-24 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}).
Productivity is tested with the following strains: *E. coli* ATCC 25922, *K. pneumoniae* ATCC 27736, *E. aerogenes* ATCC 13048, *E. faecalis* ATCC 19433, *S. pyogenes* ATCC 12384, *S. epidermidis* ATCC 12228. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.

13 - LIMITATIONS OF THE METHOD

- Nutrient Broth is not suitable for the cultivation of fastidious microorganisms and for the cultivation of anaerobes.
- Sub-culture onto suitable solid media is necessary for purification of the culture and to perform identification tests. If relevant, perform antimicrobial susceptibility testing.
- In clinical microbiology, this culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of 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 Material 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 does not 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 inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredients 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.
- 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 +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 for the validation of the shelf life of the finished products, according to the type (tubes/bottles) and the storage method (temperature and packaging). According to MacFaddin the self-prepared tubes/flasks may be stored at +2°C /+8°C for 6 months.[7]

16 - REFERENCES

1. AOAC (1995) Bacteriological Analytical Manual. 8th ed.
2. AOAC (1995) Official methods of analysis of AOAC International, 16th ed.
3. APHA (1975) Standard Methods for Examination of Water and Wastewater 14th ed.
4. APHA (1992) Compendium of methods for the microbiological examination of foods, 3rd ed.
5. APHA (1993) Standard methods for the microbiological examination of dairy products, 16th ed.
6. APHA (1995) Standard methods for the examination of water and wastewater, 19th ed.
7. 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	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	2020/09
Revision 2	Update of “intended use”, “test procedure”, “precautions and warnings” and “storage conditions and shelf life	2022/05
Revision 3	Update of chapters 1, 3, 9, 13, 14, 15, 16	2023/03

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

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