INSTRUCTIONS FOR USE

TRYPTOSE (BIOTONE) BROTH

Dehydrated culture medium

Tryptose (Biotone) Broth - from the left: un-inoculated tube, growth of *S.aureus*

1 - INTENDED USE

In vitro diagnostic. General purpose medium for the cultivation of nutritionally fastidious microorganisms.

2- COMPOSITION

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

Tryptose (Biotone)	20.000 g
Glucose	1.000 g
Sodium chloride	5.000 g
Thiamine HCl	0.005 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

Tryptose (Biotone) Broth is a general purpose medium that supports the growth of a wide variety of aerobic and facultative anaerobic nutritionally fastidious microorganisms.[1,2] Tryptose (Biotone) Broth corresponds to Tryptose Vitamin B medium described in Diagnostic Procedures and Reagents APHA Manual.[3] Tryptose (formerly named Biotone by Biolife), is a mixture of enzymatic hydrolysates of proteins and is a source of carbon, nitrogen, vitamins and minerals for microbial growth; glucose is a source of energy; sodium chloride maintains osmotic balance. According to McCullough[4], thiamine HCl addition to Tryptose Broth enhances the recovery of *Brucella* species, especially *Brucella suis*. Tryptose Broth may be used for the preparation of enriched, selective, diagnostic media as described by WHO publication.[5]

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 26 g in 1000 mL of cold purified water, heat to dissolve, distribute and sterilise by autoclaving at 121 °C for 15 minutes. For specific uses, add the required enrichment.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, limpid
Final pH at 20-25°C	7.2 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Tryptose (Biotone) Broth	Dehydrated medium	4011462 4011464	500 g (19.2) 5 kg (192)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops, needles and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the cultures.

8 - SPECIMENS

Tryptose (Biotone) Broth may be inoculated with a variety of clinical[1] and non-clinical[6] specimens for the cultivation/enrichment of microorganisms or with colonies cultivated on other isolation media. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.

9 - TEST PROCEDURE

With a bacteriological needle or loop inoculate the liquid medium in a test tube with the specimen or with a colony grown on another isolation medium. Incubate at the temperature and for the time required by laboratory procedures. Usually, an incubation temperature of 35 ± 2°C for 18-24 hours is adequate for cultivation of common aerobes and facultative anaerobes.
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 the growths are 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

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compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of un-supplemented medium.

CONTROL STRAINS			INCUBATION T°/ t / ATM	EXPECTED RESULTS
S.aureus	ATCC	25923	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

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Tryptose (Biotone) Broth REF 401146, is tested for productivity 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 organisms in test tubes, incubating at 35-37°C 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: *P.aeruginosa* ATCC 14207, *S.aureus* ATCC 25923, *M.luteus* ATCC 9341, *E.faecalis* ATCC 19433, *S.pyogenes* ATCC 19615, *S.pneumoniae* ATCC 6301, *N.gonorrhoeae* ATCC 19424, *C.albicans* ATCC 18804, *A.brasiliensis* ATCC 9642. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.

13 - LIMITATIONS OF THE METHOD

- The nutritional requirements of microorganisms can be different, it is therefore possible that some microbial strains do not grow or grow scantily.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.
- The preparation of enriched, selective diagnostic media with the addition of specific compounds[5] must be validated by the user.
- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- 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 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 +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).

16 – REFERENCES

1. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
2. Atlas R, Parks LC. Handbook of Microbiological Media. 2nd edition.n. CRC Press,1997
3. Diagnostic Procedures and Reagents, 3rd Edition, APHA, New York; 1970.
4. McCullough WG, Mills RL, Herbst EJ, Roessler WJ and Brewer CR, J Bacteriol1947; 53:
5. OMS. La Brucellose: Techniques de Laboratoires. Serie de Monographies, 1968, N. 55
6. Standard Methods for the Microbiological Examination of Dairy Products, 9th Ed., APHA, New York. 1948

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
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2022/03
Revision 2	Removal of obsolete classification	2023/04

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

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