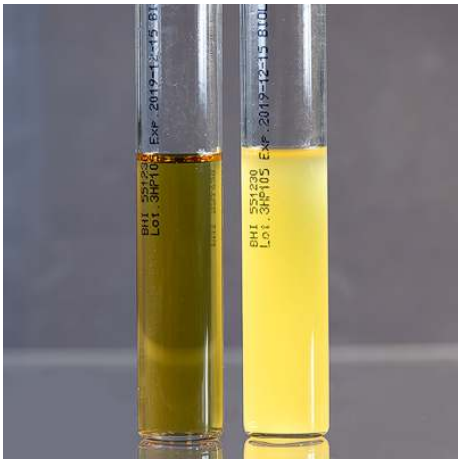
**INSTRUCTIONS FOR USE**

# BRAIN HEART INFUSION BROTH

**Dehydrated culture medium**



Brain Heart Infusion Broth:
from left: un-inoculated tube, growth of S.*aureus*

## 1 - INTENDED USE

*In vitro* diagnostic. General purpose liquid medium for the cultivation of fastidious and non-fastidious microorganisms, including aerobic and anaerobic bacteria and fungi from a variety of clinical specimens.

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

| | |
|---|---|
| Dehydrated brain infusion | 12.5 g |
| Dehydrated heart infusion | 5.0 g |
| Enzymatic digest of animal tissues | 10.0 g |
| Glucose | 2.0 g |
| Sodium chloride | 5.0 g |
| Disodium hydrogen phosphate | 2.5 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

Brain Heart Infusion (BHI) Broth is based on the formula proposed in 1919 by Edward Rosenow[1] and later modified in 1923 by Russell Haden[2]. Modern BHI Broth typically uses a dried infusions from brain and heart, rather than calf brain tissue, and uses disodium phosphate as a buffer, rather than the calcium carbonate used by Rosenow and Haden.
BHI Broth is a general purpose, nutritionally rich medium for the cultivation and maintenance of a variety of fastidious and non-fastidious microorganisms, including aerobic and anaerobic bacteria, yeasts and moulds, from clinical and non-clinical specimens using suitable incubation temperatures and time[3]. BHI Broth is used for the preparation of staphylococcal broth culture for performing coagulase test.[4]
BHI Broth can be used to start the culture process for urease test of *H.pylori*.[5,6]
Brain and heart infusions and peptone are sources of nitrogen, carbon, vitamins and minerals for microbial growth; glucose provides an energy source, sodium chloride maintains osmotic balance, dibasic sodium phosphate is included as a buffer system.

## 4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 37 g in 1000 mL of cold purified water; heat to dissolve, distribute and sterilize by autoclaving at 121°C for 15 minutes

## 5 - PHYSICAL CHARACTERISTICS

| | |
|---|---|
| Dehydrated medium appearance | beige, fine, homogeneous, free-flowing powder |
| Solution appearance | gold-yellow, limpid |
| Final pH at 20-25 °C | 7.4 ± 0.2 |

## 6 - MATERIALS PROVIDED – PACKAGING

| Product | Type | REF | Pack |
|---|---|---|---|
| Brain Heart Infusion Broth | Dehydrated medium | 4012302<br>4012304 | 500 g (13.5)<br>5 kg (135 L) |

## 7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

## 8 - SPECIMENS

Brain Heart Infusion Broth can be used for the sub-culture of colonies grown on primary isolation media. It can also be inoculated with a variety of clinical and non-clinical samples following the procedures described in the literature.[6] Good laboratory practices for collection, transport and storage of clinical specimens should be applied. Collect specimens before antimicrobial therapy where possible.

## 9 - TEST PROCEDURE

With a bacteriological needle or loop inoculate the liquid medium in a test tube or bottle with a colony grown on a plating medium or with one or two drops of the specimen, if liquid, using a sterile pipette. Swab specimens may be inserted into broth after inoculation of plated media. 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.

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## 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.[7]

| 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 Brain Heart Infusion 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 30-35°C or at 20-25°C for 18-24 hours or for 24-72 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: *S.aureus* ATCC 25923, *S.pyogenes* ATCC 19615, *S.pneumoniae* ATCC 6301, *E.faecalis* ATCC 19433, *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.
- 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.
- 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.
- Apply Good Manufacturing Practice in the preparation process of tubed or bottled 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.
- 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 period of validity of the finished products, according to the type (tubes/bottles), and the storage method applied (temperature and packaging).

## 16 - REFERENCES

1. Rosenow EC. Studies on elective localization. J Dent Research 1919; 1:205-49.
2. Hayden RL. Elective localization in the eye of bacteria from infected teeth. Arch Int Med1923; 32:828-49.
3. Atlas R, Snyder J. Reagents, Stains and Media: Bacteriology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019
4. ISO 6888-1:1999 Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). Part 1: Technique using Baird-Parker agar medium.
5. Public Health England- UK Standards for microbiology investigations (UK SMI) B55. Investigation of infectious causes of dyspepsia. Issue no: 7; 03.10.2019
6. McElvania E, Singh K. Specimen Collection, Transport and Processing: Bacteriology . In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
7. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004.

**TABLE OF APPLICABLE SYMBOLS**

| | | | | |
|---|---|---|---|---|
| **REF** or **REF** Catalogue number | **LOT** Batch code | **IVD** *In vitro* Diagnostic Medical Device | Manufacturer | Use by |
| Temperature limitation | Σ 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 3 | Updated layout and content | 2020/09 |
| Revision 4 | Point 2: detailed description of brain and heart infusions and peptone | 2020/12 |
| Revision 5 | Modification of “precautions and warnings” and “storage conditions and shelf life”. | 2022/01 |
| Revision 6 | Removal of obsolete classification | 2023/04 |

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

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