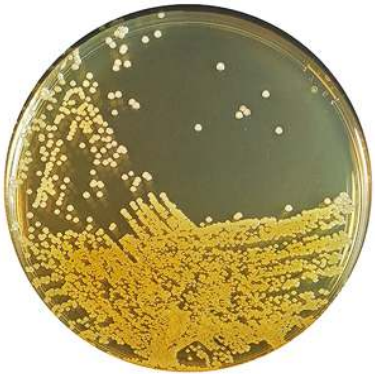
INSTRUCTIONS FOR USE

BRAIN HEART INFUSION AGAR

Dehydrated culture medium



S.aureus on Brain Heart Infusion Agar

1 - INTENDED USE

In vitro diagnostic. General purposes medium for the cultivation and maintenance of fastidious and non-fastidious microorganisms, from a variety of clinical and non-clinical specimens.

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

Brain heart infusion and peptones	27.5 g
Glucose	2.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate	2.5 g
Agar	15.0 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) Agar is based on the formula proposed in 1919 by Edward Rosenow[1] and later modified in 1923 by Russell Haden[2]. Modern BHI Agar typically uses a dried infusion from porcine 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 Agar 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 and fungi from clinical and non-clinical specimens[3]. With the addition of antimicrobials such as penicillin and streptomycin[4] or cycloheximide and chloramphenicol[5], the medium may be used for selective isolation of pathogenic fungi as the growth of bacteria and many saprophytic fungi is inhibited or delayed. Supplemented with antimicrobials and blood, it supports the growth of the tissue phase of *Histoplasma capsulatum* and other pathogenic fungi such as *Coccidioides immitis*.[6,7]

Brain heart infusion and peptones 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. Because BHI Agar contains glucose at a concentration of 0.2%, it is not useful for bacterial haemolysis detection.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 52 g in 1000 mL of cold distilled water; heat to boiling with frequent agitation and autoclave at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes. BHI Agar can also be distributed in tubes before sterilization.

5 - PHYSICAL CHARACTERISTICS

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

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Brain Heart Infusion Agar	Dehydrated medium	4012352	500 g (9,6L) CND W0104010101; EDMA: 14.01.01.01; RDM: 1920102/R
		4012354	5 kg (96 L) CND W0104010101; EDMA: 14.01.01.01; RDM: 1920106/R

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 - SPECIMENS

Brain Heart Infusion Agar can be used in plate for the sub-culture of colonies grown on primary isolation media, for the purification of the colonies or in tubes for the maintenance of the cultures. It can also be inoculated with a variety of clinical and non-clinical samples following the procedures described in the literature.[8,9] Good laboratory practices for collection, transport and storage of clinical specimens should be applied. Collect specimens before antimicrobial therapy where possible.

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

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 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.

Incubate in aerobic or anaerobic atmosphere at 35-37°C for at least 48 hours or in duplicate in air at 25 ± 2°C and 35 ± 2°C for 48 hours or more. 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. Consult the procedures outlined in the references for further information.[8,9]

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: mktg@biolifeitaliana.it; export@biolifeitaliana.it; web: www.biolifeitaliana.it

10 - READING AND INTERPRETATION

The presence of microorganisms is indicated by the appearance of colonies of varying morphology and size. The characteristics of the growths are closely related to the type or types of cultivated microorganisms.

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
S.aureus ATCC 25923	37°C / 24H / A	good growth
C.albicans ATCC 18805	25°C / 72H / 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 Agar is tested for productivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by semi-quantitative ecometric technique, with 9 bacterial strains by incubating at 32-35°C for 18-24 hours: *S.flexneri* ATCC 12022, *K.rhizophila ATCC 9341, L.monocytogenes ATCC 13932, N,gonorrhoeae ATCC 43069, P.aeruginosa* ATCC 27853, *S.aureus* ATCC 6538, *S.epidermidis* ATCC 12228, *S.pneumoniae* ATCC 6305, *S.pyogenes* ATCC 12384 and 2 mycological strains by incubating at 25-30°C for 68-72 hours: *C.albicans* ATCC 18804 and *A.brasiliensis* ATCC 9642. After incubation all strains show a good growth in both tested batches.

13 - LIMITATIONS OF THE METHOD

- If BHI Agar is used for the inoculation of non-sterile clinical specimens, a selective medium should also be streaked to avoid overgrowth by contaminating organisms.
- The nutritional requirements of microorganisms can be different, it is therefore possible that some microbial strains do not grow or grow scantily.
- 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.
- 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.
- 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 were damaged or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

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. Media Reagents and Stains. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. Ajello, Georg, Kaplan and Kaufman. 1963. CDC laboratory manual for medical mycology. PHS Publication No. 994, U.S. Government Printing Office, Washington, D.C.
6. Howell A. Public Health Reports 1948; 63:173-178.
7. Creitz JR, Puckett TF. A Method for Cultural Identification of Coccidioides Immitis. Amer J Clin Path 1954; 24:1318-1323.

8. Baron EJ, Specimen Collection, Transport and Processing:Bacteriology. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.270
9. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). Content current as of: 02/21/2020

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 3	Updated layout and content	2020/05

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

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E-mail: mktg@biolifeitaliana.it; export@biolifeitaliana.it; web: www.biolifeitaliana.it