



BHI Broth

M210I

Intended use:

Recommended for enrichment of pathogenic cocci; *Staphylococcus aureus* from food, animal feeding stuffs, milk and milk products. The composition and performance criteria of this medium are as per the specifications laid down in ISO 6888-1:2021, ISO 8870:2006 and ISO 11133:2014 /Amd.2 :2020 (E).

Composition**

ISO specification -Brain Heart Infusion Broth

Ingredients	g / L
Enzymatic digest of animal tissues	10.000
Dehydrated calf brain infusion	12.500
Dehydrated beef heart infusion	5.000
Glucose	2.000
Sodium chloride	5.000
Disodium hydrogen phosphate	2.500
Final pH after sterilization (at 25°C)	7.4±0.2

BHI Broth

Ingredients	g / L
Peptone #	10.000
HM infusion, solids ##	12.500
BHI Powder \$	5.000
Glucose (Dextrose)	2.000
Sodium chloride	5.000
Disodium hydrogen phosphate	2.500
Final pH after sterilization (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Enzymatic digest of animal tissues

Equivalent to Dehydrated calf brain infusion

\$ Equivalent to Dehydrated beef heart infusion

Directions

Suspend 37.0 gram in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense into bottles or tubes as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. For best results, the medium should be used on the day it is prepared, otherwise, it should be boiled or steamed for a few minutes and then cooled before use.

Principle And Interpretation

Rosenow (1) devised the original Brain Heart Infusion Broth by adding brain tissue to dextrose broth. BHI Broth is a highly nutritious medium and is also well buffered to support the growth of wide variety of microorganisms (2-4). Recently this medium has been recommended by ISO committee for the detection of *Staphylococcus aureus* (5,6,7). The With the additions of desired additives this medium can be specifically adopted for cultivation of various bacteria. BHI Broth is also used for the preparation of inocula for use in antimicrobial susceptibility tests.

Type of specimen

Food samples and animal feeding stuffs; Dairy samples

Specimen Collection and Handling:

For food samples follow appropriate techniques for handling specimens as per established guidelines (5,6).

For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7-8).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Processing : ISO 8870 / IDF 83-2006

Inoculum preparation : Typical colony isolated on Baird Parker Agar (M043I) to be inoculated in BHI Broth and incubate at 35-37 °C for 24 h ± 2 h.

Confirmation (Coagulase Test) : Aseptically add 0,1 ml of each culture from BHI Broth (M210I) to 0,3 ml of the rabbit plasma in sterile haemolysis tubes or bottles and incubate at 35-37 °C. Examine for clotting of the plasma after 4 h to 6 h of incubation, if the test is negative, re-examine at 24 h of incubation. Consider the coagulase test to be positive if the volume of clot occupies more than half of the original volume of the liquid.

Warning and Precautions

Read the label before opening the pack. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light amber coloured, clear to slightly opalescent solution

Reaction

Reaction of 3.7% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth
Productivity		
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	50-100	good -luxuriant

Key: (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

1. Rosenow, 1919, J. Dental Res., 1:205.
2. Conant N.F., 1950, Diagnostic Procedures and Reagents, 3rd Ed., APHA, Inc., New York, p. 452.
3. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
4. Roseburg T. et al, 1944, J. Inf. Dis., 74:131.
5. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase positive Staphylococci (*Staphylococcus aureus* and other species). International Organization for Standardization (ISO), 1999 Ammd 2:2018-07, Draft ISO/DIS 6888-1.
6. Microbiology of food, animal feeding stuffs and water- Preparation, production, storage and performance culture media, EN ISO 11133:2014 /Amd.2 :2020 (E) .

7. Milk and milk-based products - Detection of thermonuclease produced by coagulase-positive Staphylococci. International Organization for Standardization (ISO), 8870 / IDF 83-2006.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
9. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
10. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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Disclaimer :

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