



QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

Brain Heart Infusion Agar is a general purpose culture medium which supports the growth of a wide variety of bacterial and fungal species including many types of pathogens, such as streptococci and pneumococci.

II PERFORMANCE TEST PROCEDURE

1. Liquefy the deeps by heating in boiling water. Cool to 45–50 °C and pour into sterile Petri dishes. Allow to harden for a minimum of 30 min.
2. Inoculate representative samples with the cultures listed below.
 - a. Using a 0.01 mL calibrated loop, inoculate the plate, slant and bottle samples using 10⁻¹ dilutions of 18- to 24-h **Trypticase™** Soy Broth cultures.
 - b. Incubate strains (with loosened caps) at 35 ± 2 °C in an aerobic atmosphere.
3. Examine plates or slants after 18–24 and 48 h for amount of growth.
4. Expected Results

Organisms	ATCC®	Recovery
* <i>Candida albicans</i>	10231	Moderate to heavy growth
* <i>Shigella flexneri</i>	12022	Moderate to heavy growth
* <i>Streptococcus pneumoniae</i>	6305	Moderate to heavy growth

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Incubate uninoculated representative tubes at 20–25 °C and 30–35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Brain Heart Infusion (BHI) Agar is a general purpose medium suitable for the cultivation of a wide variety of organism types, including bacteria, yeasts and molds. With the addition of 10% sheep blood, it is used for the isolation and cultivation of a wide variety of fungal species, including systemic fungi, from clinical and nonclinical sources.^{1,2}

V SUMMARY AND EXPLANATION

In the early years of bacteriology, meat infusions were utilized as the growth-supporting components in a large number of culture media. Although they were cumbersome to prepare, lacked consistency from batch to batch and were undefined as to their nutritive content, they enabled the cultivation of microorganisms in both solid and liquid media. As the state-of-the-art in enzymology and chemistry advanced, methods were developed for the preparation of peptones which were the result of enzymatic or acid hydrolysis of animal tissues or products and vegetable substances. These peptones currently are the major nutritional additives to culture media formulations, but infusions are still utilized in specific media.

Brain Heart Infusion Agar is one formulation in which meat infusion is used, although, unlike in the earlier days, the infusion components are solids resulting from the drying of the liquid infusion material rather than the liquid components themselves. Two peptones are also included as sources of nutrients.

This medium has proven to be effective in the cultivation of a wide variety of microorganisms, including many types of pathogens. BHI Agar currently is recommended as a general medium for aerobic bacteriology and for the primary recovery of fungi from clinical specimens.²

VI PRINCIPLES OF THE PROCEDURE

BHI Agar derives its nutrients from the brain heart infusion, peptone and dextrose components. The peptones and infusion are sources of organic nitrogen, carbon, sulfur, vitamins and trace substances. Dextrose is the carbohydrate source that microorganisms utilize by fermentative action. The medium is buffered through the use of disodium phosphate.

VII REAGENTS

Brain Heart Infusion Agar

Approximate Formula* Per Liter Purified Water

Brain Heart, Infusion from (solids).....	8.0 g	Dextrose.....	2.0 g
Peptic Digest of Animal Tissue.....	5.0 g	Disodium Phosphate.....	2.5 g
Pancreatic Digest of Casein.....	16.0 g	Agar.....	13.5 g
Sodium Chloride.....	5.0 g		

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–25 °C. Avoid freezing and overheating. Do not open until ready to use. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Minimize exposure to light.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{3,4} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Brain Heart Infusion Agar

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Prepare plated medium from the agar deeps by liquefying the medium in boiling water, cooling to 45–50 °C and pouring into sterile Petri dishes. Additives, e.g., blood, can be used as desired.

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora.

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. Since many pathogens require carbon dioxide on primary isolation, plates of plain BHI agar may be incubated in an atmosphere containing approximately 5% CO₂. Incubate plates at 35 ± 2 °C for 18–24 h.

For isolation of fungi from potentially contaminated specimens, a selective medium should be inoculated along with the nonselective medium. Incubate the plates at 25–30 °C in an inverted position (agar side up) with increased humidity. For isolation of fungi causing systemic mycoses, two sets of media should be inoculated, with one set incubated at 25–30 °C and a duplicate set at 35 ± 2 °C. All cultures should be examined at least weekly for fungal growth and should be held for 4–6 weeks before being reported as negative.

BHI Agar Slants are primarily used for the cultivation and maintenance of pure cultures of microorganisms.

User Quality Control: See “Quality Control Procedures.”

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory's standard quality control procedures.

X RESULTS

Slant cultures may be used as sources of inocula for studies or for organism maintenance purposes.

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.³⁻⁵

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Brain Heart Infusion Agar are tested for performance characteristics. Using a 0.01 mL calibrated loop, representative samples of the lot are streak-inoculated with **Trypticase** Soy Broth cultures diluted 10-1 of *Candida albicans* (ATCC 10231), *Shigella flexneri* (ATCC 12022) and *Streptococcus pneumoniae* (ATCC 6305). Inoculated containers are incubated at 35 ± 2 °C with loosened caps. Containers are read for growth after 18–24 h, and 42–48 h incubation. All cultures show visible growth within 18–24 h and moderate to heavy growth after 42–48 h.

XIII AVAILABILITY

Cat. No. Description

221610 **BD BBL™** Brain Heart Infusion Agar Slants, Pkg. of 10 size K tubes

XIV REFERENCES

1. Creitz, J.R., and T.F. Puckett. 1954. A method for cultural identification of *Coccidioides immitis*. Am. J. Clin. Pathol. 24:1318-1323.
2. Merz, W.G., and G.D. Roberts. 1995. Detection and recovery of fungi from clinical specimens, p. 709-722. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Tenover (ed.) 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
4. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Baily & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
5. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.

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