

**QUALITY CONTROL PROCEDURES****I INTRODUCTION**

Brain Heart Infusion Agar with 10% Sheep Blood is used for the cultivation of pathogenic fungi and nonpathogenic fungi from a variety of clinical and nonclinical specimens.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
 - a. Streak the plates for isolation using fresh fungal agar cultures for all fungal organisms. For *C. albicans* streak inoculate using a 0.01 mL loop diluted to yield $10^3 - 10^4$ CFU/0.01 mL.
 - b. Incubate plates at 25 ± 2 °C in an aerobic atmosphere.
 - c. Include plates of a previously tested lot of Brain Heart Infusion Agar with 10% Sheep Blood or Sabouraud Dextrose Agar as controls for all strains.
2. Examine plates up to 7 days for amount of growth and pigmentation.
3. Expected Results

Organisms	ATCC™	Recovery
* <i>Candida albicans</i>	60193	Fair to heavy growth.
* <i>Trichophyton mentagrophytes</i>	9533	Fair to heavy growth.
<i>Blastomyces dermatitidis</i>	56218	Fair to heavy growth. Colonies white and cottony.
<i>Cryptococcus neoformans</i>	32045	Fair to heavy growth. Colonies initially wrinkled and whitish. May become mucoid and cream to brown.
<i>Penicillium roquefortii</i>	9295	Fair to heavy growth. Colonies powdery, initially white but may become green to blue

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.4 ± 0.2 .
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 35 ± 2 °C in an aerobic atmosphere for 72 h and examine for microbial contamination.

PRODUCT INFORMATION**IV INTENDED USE**

Brain Heart Infusion Agar with 10% Sheep Blood is used for the isolation and cultivation of a wide variety of fungal species, including systemic fungi,¹ from clinical and nonclinical sources. The plates are deep-filled to reduce the effects of drying during prolonged incubation.

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. It has served as the base medium for new culture media formulations when supplemented with sheep blood, cycloheximide, chloramphenicol, and/or gentamicin. BHI Agar is recommended as a general medium for aerobic bacteriology and for the primary recovery of fungi from clinical specimens.^{2,3} Brain Heart Infusion Agar with 10% Sheep Blood can be used to isolate systemic fungi which may grow poorly on the nonenriched medium.^{2,3}

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 which microorganisms utilize by fermentative action. The medium is buffered through the use of disodium phosphate. The defibrinated sheep blood provides essential growth factors for the more fastidious fungal organisms.

VII REAGENTS

Brain Heart Infusion Agar with 10% Sheep Blood

Approximate Formula* Per Liter Purified Water

Brain Heart, Infusion from (solids)	8.0 g
Peptic Digest of Animal Tissue	5.0 g
Pancreatic Digest of Casein.....	16.0 g
Sodium Chloride.....	5.0 g
Dextrose.....	2.0 g
Disodium Phosphate.....	2.5 g
Agar.....	13.5 g
Sheep Blood, defibrinated.....	10%

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

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

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens.

"Standard Precautions"⁴⁻⁷ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2 – 8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 – 8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times including up to 6 weeks for mycology media. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking 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.^{8,9}

Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Brain Heart Infusion Agar with 10% Sheep Blood (Deep Fill)

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

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Streak the specimen as soon as possible after it is received in the laboratory using a sterile inoculating loop to obtain isolated colonies.

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 in an aerobic atmosphere.

All cultures should be examined at least weekly for fungal growth and should be held for 4 – 6 weeks before being reported as negative.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Examine plates for fungal colonies exhibiting typical color and morphology. Biochemical tests and serological procedures should be performed to confirm findings.

XI LIMITATION 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.⁸⁻¹²

XII AVAILABILITY

Cat. No.	Description
221843	BBL™ Brain Heart Infusion Agar with 10% Sheep Blood (Deep Fill), Pkg. of 20 plates

XIII REFERENCES

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