



BBL™ Brain Heart Infusion CC Agar with Blood

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Brain Heart Infusion Agar with chloramphenicol, cycloheximide and sheep blood is a selective medium used for the isolation of pathogenic fungi from specimens heavily contaminated with bacteria and saprophytic fungi.

II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with the cultures listed below.
 - For *E. coli* and *C. albicans*, streak inoculate 1 µL (0.001 mL) from a 4 – 5 h culture of **Trypticase™** Soy Broth diluted to yield $10^6 - 10^7$ CFU/mL.
 - For all other organisms, inoculate directly from a stock plate using a fresh fungal culture (up to one month in age).
- Incubate all plates at 20 – 25°C.
- Examine at intervals up to 7 days for growth, colony color and selectivity.
- Expected Results

Organisms	ATCC™	Recovery	Colony Color
<i>Aspergillus brasiliensis</i>	16404	Inhibition (partial to complete)	N/A
* <i>Blastomyces dermatitidis</i>	56218	Fair to heavy growth	White
<i>Candida albicans</i>	10231	Fair to heavy growth	White
* <i>Escherichia coli</i>	25922	Inhibition (partial to complete)	N/A
<i>Trichophyton mentagrophytes</i>	9533	Fair to heavy growth	N/A

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- Examine plates as described under "Product Deterioration."
- Visually examine representative plates to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for the adherence to the specification 7.4 ± 0.2 .
- Note the firmness of plates during the inoculation procedure.
- Incubate uninoculated representative plates at 33 – 37°C and 20 – 25°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

This medium is used in qualitative procedures for isolation and cultivation of pathogenic and nonpathogenic fungi from clinical and nonclinical specimens. An increased fill volume is incorporated to reduce the effects of drying during prolonged incubation.

V SUMMARY AND EXPLANATION

Brain Heart Infusion (BHI) Agar is a general purpose medium suitable for the primary recovery of fungi.¹ The addition of sheep blood is recommended to improve the recovery of pathogenic dimorphic fungi.²

Antimicrobial agents, including chloramphenicol in combination with cycloheximide (CC), are employed to improve the recovery of pathogenic fungi from specimens heavily contaminated with bacteria and saprophytic fungi.²

VI PRINCIPLES OF THE PROCEDURE

BHI Agar is an enriched medium consisting of infusions of brain and heart tissue, peptones and dextrose to supply the nutrients necessary to support the growth of fungi. Defibrinated sheep blood supplies additional nutrients to support the isolation and cultivation of dimorphic species.

Supplementing the medium with antimicrobial agents increases the recovery of pathogenic fungi from clinical specimens by inhibiting bacteria and saprophytic fungi. Chloramphenicol is a broad spectrum antibiotic that inhibits both gram-positive and gram-negative bacteria. Cycloheximide is an antifungal agent that is primarily active against saprophytic fungi and does not inhibit yeasts or dermatophytes.

VII REAGENTS

BHI Agar Base

Approximate Formula* Per Liter Purified Water

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

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

BHI CC Agar with Blood

Approximate Formula* Per Liter Purified Water

BHI Agar Base	52.0 g
Cycloheximide	0.5 g
Chloramphenicol.....	50.0 mg
Sheep Blood, defibrinated	5%

*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"³⁻⁶ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. Prior to discarding, sterilize prepared plates, specimen containers and other contaminated materials by autoclaving.

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 up to 6 weeks. 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

Refer to appropriate texts for details of specimen collection and handling procedures.^{2,7-9}

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 CC Agar with Blood.

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.

Inoculate the medium as soon as the specimen arrives at the laboratory. Streak the specimen onto the medium with a sterile inoculating loop to obtain isolated colonies. Consult appropriate references for information about the processing and inoculation of specimens such as tissues, skin scrapings, hair, nail clippings, etc.^{8,10-13}

For isolation of fungi causing cutaneous mycoses, a general-purpose, non-selective medium should be inoculated along with a selective medium. Incubate the plates at 25 – 30°C and a duplicate set at 35 – 37°C in an inverted position (agar-side up) with increased humidity. 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 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 pathogenic species exhibiting typical color and morphology. Gram staining, biochemical tests and other procedures should be performed to confirm findings.

XI LIMITATIONS OF THE PROCEDURE

This prepared plated medium is intended for primary isolation. Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical tests and other identification procedures. Consult appropriate references for further information.^{8,10-14}

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. The agents in selective media may inhibit some strains of the desired species or permit growth of a species they were designed to inhibit, especially if the species is present in large numbers in the specimen. Specimens cultured on selective media should, therefore, also be cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII AVAILABILITY

Cat. No.	Description
296178	BBL™ Brain Heart Infusion CC Agar with Blood, Pkg. of 20 plates

XIII REFERENCES

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Becton, Dickinson and Company
 7 Loveton Circle
 Sparks, MD 21152 USA
 800-638-8663
www.bd.com/ds

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