



BBL™ Brain Heart Infusion Agar with 5% Sheep Blood
BBL™ Brain Heart Infusion Agar with 10% Sheep Blood,
Chloramphenicol and Gentamicin
BBL™ Brain Heart CC Agar with 10% Sheep Blood and Gentamicin
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QUALITY CONTROL PROCEDURES

I INTRODUCTION

These media are used in qualitative procedures for the isolation and cultivation of pathogenic and nonpathogenic fungi from clinical and nonclinical specimens.

II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with the cultures listed below.
 - For *C. albicans*, *E. coli* and *S. aureus*, streak inoculate 1 µL (0.001 mL) from a 4 – 5 h culture of **Trypticase™** Soy Broth diluted to yield 106 – 107 CFU/mL.
 - For remaining organisms, inoculate directly from a stock plate using a fresh fungal culture (up to one month in age).
 - Include Sabouraud Dextrose Agar controls for all organisms.
- Incubate all tubes at 20 – 25 °C.
- Examine at intervals up to 7 days for growth, colony color and selectivity.
- Expected Results

Medium	Organisms	ATCC®	Recovery	Colony Color
BHI Sheep Blood Agar	<i>Blastomyces dermatitidis</i>	56218	Fair to heavy growth	White to cream to tan
	* <i>Candida albicans</i>	60193	Fair to heavy growth	White to cream
	* <i>Trichophyton mentagrophytes</i>	9533	Fair to heavy growth	White to cream/yellow
BHI Sheep Blood Agar with Chloramphenicol and Gentamicin	<i>Blastomyces dermatitidis</i>	56218	Fair to heavy growth	White to cream to tan
	* <i>Candida albicans</i>	10231	Fair to heavy growth	White to cream
	* <i>Escherichia coli</i>	25922	Inhibited	N/A
	* <i>Staphylococcus aureus</i>	25923	Inhibited	N/A
	* <i>Trichophyton mentagrophytes</i>	9533	Fair to heavy growth	White to cream/yellow
Brain Heart CC Sheep Blood Agar with Gentamicin	<i>Aspergillus brasiliensis</i>	16404	Inhibited	N/A
	* <i>Blastomyces dermatitidis</i>	56218	Fair to heavy growth	N/A
	* <i>Candida albicans</i>	10231	Fair to heavy growth	White to cream, pasty
	* <i>Escherichia coli</i>	25922	Inhibited	N/A
	* <i>Trichophyton mentagrophytes</i>	9533	Fair to heavy growth	White to cream/yellow, velvety

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- Examine tubes as described under “Product Deterioration.”
- Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
- For Brain Heart CC Sheep Blood Agar with Gentamicin, determine the pH potentiometrically at room temperature for adherence to the specification of 7.4 ± 0.2 .
- Incubate uninoculated representative tubes at 33 – 37 °C and 20 – 25 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

These media are used in qualitative procedures for the isolation and cultivation of pathogenic and nonpathogenic fungi from clinical and nonclinical specimens.

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.¹

Antimicrobial agents, including chloramphenicol, chloramphenicol in combination with cycloheximide (CC), and gentamicin are incorporated to improve the recovery of pathogenic fungi from specimens heavily contaminated with bacteria and saprophytic fungi.¹

VI PRINCIPLES OF THE PROCEDURE

BHI Agar consists 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 BHI Agar with antimicrobial agents increases the recovery of pathogenic fungi from clinical specimens by inhibiting bacteria. Gentamicin inhibits gram-negative bacteria. 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

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
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 Sheep Blood Agar consists of the above ingredients with 5% defibrinated sheep blood per liter.

BHI Sheep Blood Agar with Chloramphenicol and Gentamicin consists of BHI Agar with 0.05 g/L chloramphenicol, 0.05 g/L gentamicin and 10% defibrinated sheep blood per liter.

Brain Heart CC Sheep Blood Agar with Gentamicin consists of BHI Agar with 0.05 g/L chloramphenicol, 0.5 g/L cycloheximide, 0.05 g/L gentamicin and 10% sheep blood per liter.

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

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

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

"Standard Precautions"²⁻⁵ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.

Storage Instructions: On receipt, store tubes in the dark according to label directions. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labelled 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 tubes if they show evidence of microbial contamination, discoloration, precipitation, evaporation or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of specimen collection and handling procedures.^{1,6-8}

IX PROCEDURE

Material Provided: Brain Heart Infusion Agar with 5% Sheep Blood, Brain Heart Infusion Agar with 10% Sheep Blood, Chloramphenicol and Gentamicin, or Brain Heart CC Agar with 10% Sheep Blood and Gentamicin

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

Test Procedure: Observe aseptic techniques.

Inoculate the medium as soon as possible after the specimen arrives at the laboratory. Streak the specimen onto the medium with a sterile inoculating loop to obtain isolated colonies. Consult appropriate texts for information about the processing and inoculation of specimens such as tissues, skin scrapings, hair, nail clippings, etc.^{1,6-9}

For isolation of fungi that cause cutaneous mycoses, a general-purpose, nonselective medium should be inoculated along with a selective medium. Incubate one set of tubes at 25 – 30 °C and a duplicate set at 35 – 37 °C. Examine all cultures at least weekly for growth and hold cultures for 4 – 6 weeks before reporting them 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

Examine medium for growth of fungal species, noting color and morphology.

XI LIMITATIONS OF THE PROCEDURE

For identification of microorganisms, the organism must be in pure culture. Morphological, nutritional, biochemical and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.⁶⁻¹¹

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 PERFORMANCE CHARACTERISTICS

Brain Heart Infusion Agar with 5% Sheep Blood

Prior to release, all lots of Brain Heart Infusion Agar with 5% Sheep Blood are tested for specific product characteristics. Samples of the lot are tested with *Blastomyces dermatitidis* ATCC 56218, *Candida albicans* ATCC 60193 and *Trichophyton mentagrophytes* ATCC 9533, inoculated directly by streaking the surface of the medium. Tubes are incubated with loose caps at 20 – 27 °C for up to 7 days. Fair to heavy growth is observed with all the organisms.

Brain Heart CC Agar with 10% Sheep Blood and Gentamicin

Prior to release, all lots of Brain Heart CC Agar with 10% Sheep Blood and Gentamicin are tested for specific product characteristics. Samples of the lot are tested with *Blastomyces dermatitidis* ATCC 56218, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922 and *Trichophyton mentagrophytes* ATCC 9533, inoculated directly by streaking the surface of the medium (for *C. albicans* and *E. coli*, normal saline suspensions diluted to yield 10³ to 10⁴ CFU are used). Tubes are incubated with loose caps at 20 – 27 °C for up to 7 days. Fair to heavy growth is observed with *B. dermatitidis*, *C. albicans* and *T. mentagrophytes*. Partial to complete inhibition is observed with *E. coli*.

XIII AVAILABILITY

Cat. No.	Description
297199	BD BBL™ Brain Heart Infusion Agar with 5% Sheep Blood Slants Pkg. of 10 size A tubes CE
296067	BD BBL™ Brain Heart Infusion Agar with 5% Sheep Blood Slants Ctn. of 100 size A tubes
295756	BD BBL™ Brain Heart Infusion Agar with 10% Sheep Blood, Chloramphenicol and Gentamicin Slants, Ctn. of 100 size A tubes
296358	BD BBL™ Brain Heart CC Agar with 10% Sheep Blood and Gentamicin Slants Pkg. of 10 size A tubes

XIV REFERENCES

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