

Precautions⁵

1. Biosafety Level 2 practices and procedures, containment equipment and facilities are required for non-aerosol-producing manipulations of clinical specimens such as preparation of acid-fast smears. All aerosol-generating activities must be conducted in a Class I or II biological safety cabinet.
2. Biosafety Level 3 practices, containment equipment and facilities are required for laboratory activities in the propagation and manipulation of cultures of *M. tuberculosis* and *M. bovis*. Animal studies also require special procedures.

Procedure

Sterile Specimens for the Isolation of Mycobacteria¹

Normally sterile tissues may be ground in 0.2% BSA and inoculated directly in culture media. Concentrate body fluids before inoculation because they normally contain only a small number of mycobacteria. Centrifuge fluids at $\geq 3,000 \times g$ and inoculate the sediment onto liquid or solid media. For a com-

plete discussion of the inoculation of sterile specimens, refer to appropriate references.

Contaminated Specimens for the Isolation of Mycobacteria¹

A concentration of 0.2% BSA can be added to specimen sediment that has been digested and centrifuged by the N-Acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) digestion method or by using the BBL™ MycoPrep™ Mycobacterial Specimen Digestion/Decontamination Kit. Using a separate sterile pipette for each tube, add 1-2 mL of 0.2% BSA, then resuspend the sediment with the pipette or by shaking the tube gently by hand.

Several digestion procedures exist. Consult appropriate references for a complete discussion on all digestion and decontamination methods and other testing procedures.

Expected Results

All media should be examined closely for evidence of growth. Refer to the procedure established by laboratory policy or to appropriate references on typical growth patterns and confirmation tests.

Limitation of the Procedure

Bovine Albumin 5% is not recommended for use with the BACTEC™ Blood Culture System because BSA may delay detection times.¹

References

1. Merchock, Nolte and Wallace. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.). Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Davis and Dubos. 1945. J. Bacteriol. 55:11.
3. Ellinghausen and McCullough. 1962. Bacteriol. Proc. 62:54.
4. Morton, Smith, Williams and Eickenberg. 1951. J. Dent. Res. 30:415.
5. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 1999. Biosafety in microbiological and biomedical laboratories, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

Availability

Difco™ Bovine Albumin 5%

Cat. No. 266810 Prepared Tubes, 20 mL – Pkg. of 12

User Quality Control

Identity Specifications

Difco™ Bovine Albumin 5%

Appearance: Light amber, clear to very slightly opalescent.

Reaction of Solution at 25°C: pH 7.0 ± 0.2

Cultural Response

Difco™ Bovine Albumin 5%

Prepare Dubos Broth Base per label directions, substituting Bovine Albumin 5% for Dubos Medium Albumin. Inoculate and incubate at 35 ± 2°C under CO₂ for up to 3 weeks.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Mycobacterium intracellulare</i>	13950	10 ² -10 ³	Good
<i>Mycobacterium tuberculosis</i> H37Ra	25177	10 ² -10 ³	Good

Brain Heart CC Agar

Selective Brain Heart Infusion Agars

Intended Use

Brain Heart CC Agar is a selective medium used for the isolation of pathogenic fungi from specimens heavily contaminated with bacteria and saprophytic fungi.¹ It also serves as the base for enriched and more selective media supplemented with sheep blood and antibiotics.

Summary and Explanation

Brain Heart Infusion (BHI) Agar is recommended as a general-purpose medium for aerobic bacteriology and for the primary recovery of fungi from clinical specimens.^{2,3} With 10% sheep blood, it is used to isolate systemic fungi that may grow poorly on the nonenriched medium. The presence of the

antimicrobial agents, cycloheximide and/or chloramphenicol and, in modified formulations, gentamicin, penicillin and streptomycin, inhibits the growth of a wide variety of bacteria and fungi and enhances the isolation of pathogenic fungal species.

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. The addition of defibrinated sheep blood provides essential growth factors

B Brain Heart CC Agar, cont.

for the more fastidious fungal organisms. Chloramphenicol is a broad-spectrum antibiotic which inhibits a wide range of gram-positive and gram-negative bacteria. Cycloheximide inhibits most saprophytic molds. Gentamicin is an aminoglycoside antibiotic that inhibits the growth of gram-negative and some gram-positive bacteria. Penicillin primarily inhibits gram-positive bacteria. Streptomycin inhibits gram-negative organisms.

Formula

BBL™ Brain Heart CC Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	16.0	g
Brain Heart, Infusion from (solids)	8.0	g
Peptic Digest of Animal Tissue	5.0	g
Sodium Chloride	5.0	g
Dextrose	2.0	g
Disodium Phosphate	2.5	g
Cycloheximide	0.5	g
Chloramphenicol	0.05	g
Agar	13.5	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 52 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 118°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for information about the processing and inoculation of specimens.^{1,4}

For isolation of fungi from potentially contaminated specimens, a nonselective medium should be inoculated along with the selective medium. Incubate at 25-30°C (plates 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.

Expected Results

After sufficient incubation, examine cultures for fungal colonies exhibiting typical color and morphology. Biochemical tests and serological procedures should be performed to confirm findings.

Limitation of the Procedure

Some fungi may be inhibited by antibiotics in this medium.⁵

References

1. Reisner, Woods, Thomson, Larone, Garcia and Shimizu. 1999. *In* Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Kwon-Chung and Bennett. 1992. Medical mycology. Lea & Febiger, Philadelphia, Pa.
3. Chapin and Murray. 1999. *In* Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
4. Merz and Roberts. 1995. *In* Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. Ajello, Georg, Kaplan and Kaufman. 1963. CDC laboratory manual for medical mycology. PHS Publication No. 994, U.S. Government Printing Office, Washington, D.C.

User Quality Control

Identity Specifications

BBL™ Brain Heart CC Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	5.2% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to moderately hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to moderately hazy.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2

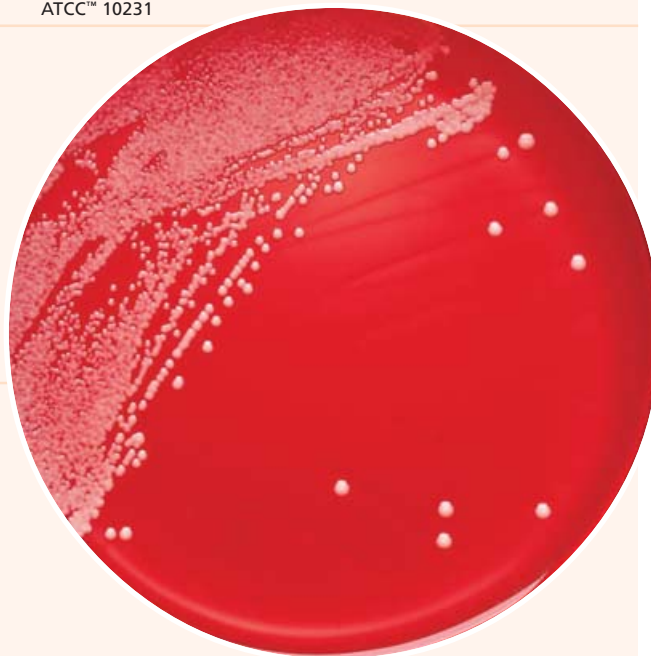
Cultural Response

BBL™ Brain Heart CC Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 25 ± 2°C under appropriate atmospheric conditions for 7 days.

ORGANISM	ATCC™	RECOVERY
<i>Aspergillus niger</i>	16404	Partial to complete inhibition
<i>Candida albicans</i>	10231	Good
<i>Escherichia coli</i>	25922	Partial to complete inhibition
<i>Trichophyton mentagrophytes</i>	9533	Good

Candida albicans
ATCC™ 10231



Availability

BBL™ Brain Heart (Infusion) CC Agar

Cat No.	211057	Dehydrated – 500 g
	296261	Prepared Plates (Deep Fill) – Pkg. of 20*
	297650	Prepared Slants (A Tubes) – Pkg. of 10*
	296106	Prepared Slants (C Tubes) – Ctn. of 100*
	221834	Mycoflask™ Bottles – Pkg. of 10*

BBL™ Brain Heart Infusion CC Agar with Sheep Blood

Cat. No.	296178	Prepared Plates (Deep Fill) – Pkg. of 20*
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BBL™ Brain Heart CC Agar with 10% Sheep Blood and Gentamicin

Cat. No.	221842	Prepared Plates (Deep Fill) – Pkg. of 10*
	296358	Prepared Slants (C Tubes) – Pkg. of 10*
	295757	Prepared Slants (C Tubes) – Ctn. of 100*

BBL™ Brain Heart Infusion Agar with 10% Sheep Blood, Gentamicin and Chloramphenicol

BS10 **CMPH** **MCM7**

Cat. No.	221841	Prepared Plates (Deep Fill) – Pkg. of 20*
	296343	Prepared Slants (C Tubes) – Pkg. of 10*
	295756	Prepared Slants (C Tubes) – Ctn. of 100*

BBL™ Brain Heart Infusion (Sheep) Blood Agar with Penicillin and Streptomycin

Cat. No.	296097	Prepared Plates (Deep Fill) – Pkg. of 20*
	297335	Prepared Slants (A Tubes) – Pkg. of 10*

*Store at 2-8°C.

Brain Heart Infusion (Broth Media)

Brain Heart Infusion • Brain Heart Infusion with Supplements • Brain Heart Infusion without Dextrose • Brain Heart Infusion Broth, Modified

Intended Use

Brain Heart Infusion (BHI) is a general-purpose liquid medium used in the cultivation of fastidious and nonfastidious microorganisms, including aerobic and anaerobic bacteria, from a variety of clinical and nonclinical materials. It serves as a base for supplemented media containing 0.1% agar, Fildes enrichment or 6.5% sodium chloride. A supplemented pre-reduced formulation in tubes is especially recommended for the cultivation of anaerobes.

Summary and Explanation

Rosenow described brain-heart infusion broth prepared by adding pieces of brain tissue to meat infusion or beef extract-dextrose broth.¹ A variation of this medium appeared for many years in the National Formulary.² The current formulation is similar to the NF Brain Heart Infusion Broth, but the brain infusion component is composed of solids resulting from the drying of the liquid material and the heart infusion component has been replaced with a peptone of partially digested animal tissue.

BHI broth is used for the cultivation of a wide variety of microorganisms, including bacteria, yeasts and molds.

BHI broth, 0.5 mL per tube, is used for the cultivation of bacteria employed in the preparation of inocula for microdilution minimal inhibitory concentration (MIC) and identification (ID) test panels. When a large number of cells are inoculated into the small volume of broth, a bacterial culture rapidly reaches its stationary phase of growth.³ The medium is also used in 5-mL amounts per tube for the preparation of inocula in antimicrobial susceptibility test procedures. This volume and the 8-mL tubes also can be used for general purposes.

Fildes enrichment may be incorporated for the growth of fastidious organisms. With the addition of 0.1% agar, the medium is used for the cultivation of anaerobes. The medium pre-reduced in Hungate tubes is recommended for the cultivation of anaerobic microorganisms, particularly obligate anaerobes.

The broth medium that contains 6.5% sodium chloride is used to differentiate the enterococci from nonenterococcal group D streptococci by the 6.5% salt tolerance test.⁴

Brain Heart Infusion without Dextrose is a basal medium used with carbohydrates for fermentation studies.

Brain Heart Infusion, Modified differs from other formulations by the quantities of the ingredients and the substitution of pancreatic digest of casein for pancreatic digest of gelatin.

Principles of the Procedure

BHI Broth is a nutritious, buffered culture medium that contains infusions of brain and heart tissue and peptones to supply protein and other nutrients necessary to support the growth of fastidious and nonfastidious microorganisms. In the formulation containing 6.5% sodium chloride, the salt acts as a differential and/or selective agent by interfering with membrane permeability and osmotic and electrokinetic equilibria in salt-intolerant organisms. Fildes enrichment (peptic digest of sheep blood) is incorporated into one tubed formulation for the cultivation of fastidious microorganisms, such as *Haemophilus influenzae*.^{5,6} The addition of 0.1% agar aids in the cultivation of anaerobic microorganisms because its consistency yields conditions of reduced oxygen tension. The pre-reduced medium in Hungate tubes is based on Hungate methods of culturing anaerobic microorganisms outside of an anaerobic chamber.⁷ The tubes provide a reduced medium in

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Bacto™ Brain Heart Infusion

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 3.7%	
Solution at 25°C:	pH 7.4 ± 0.2

Difco™ Brain Heart Infusion without Dextrose

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 3.5%	
Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Bacto™ Brain Heart Infusion or Difco™ Brain Heart Infusion without Dextrose

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Neisseria meningitidis</i>	13090	10 ² -10 ³	Good
<i>Streptococcus pneumoniae</i>	6305	10 ² -10 ³	Good
<i>Streptococcus pyogenes</i>	19615	10 ² -10 ³	Good

a self-contained, anaerobic tube sealed using a Hungate screw cap. The cap contains a butyl rubber septum stopper that permits inoculation and incubation without exposing the medium to air.

Formulae

Bacto™ Brain Heart Infusion

Approximate Formula* Per Liter	
Calf Brains, Infusion from 200 g	7.7 g
Beef Heart, Infusion from 250 g	9.8 g
Proteose Peptone	10.0 g
Dextrose	2.0 g
Sodium Chloride	5.0 g
Disodium Phosphate	2.5 g

BBL™ Brain Heart Infusion

Approximate Formula* Per Liter	
Brain Heart, Infusion from (solids)	6.0 g
Peptic Digest of Animal Tissue	6.0 g
Pancreatic Digest of Gelatin	14.5 g
Dextrose	3.0 g
Sodium Chloride	5.0 g
Disodium Phosphate	2.5 g

Difco™ Brain Heart Infusion without Dextrose

Approximate Formula* Per Liter	
Calf Brains, Infusion from 200 g	7.7 g
Beef Heart, Infusion from 250 g	9.8 g
Proteose Peptone	10.0 g
Sodium Chloride	5.0 g
Disodium Phosphate	2.5 g

Identity Specifications

BBL™ Brain Heart Infusion

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.7% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 3.7%	
Solution at 25°C:	pH 7.4 ± 0.2

BBL™ Brain Heart Infusion Broth, Modified

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.8% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 3.7%	
Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

BBL™ Brain Heart Infusion or BBL™ Brain Heart Infusion Broth, Modified

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for 7 days (incubate *C. albicans* at 20-27°C).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY BHI	RECOVERY BHI, MODIFIED
<i>Bacteroides fragilis</i>	25285	≤10 ⁴	Good	Good
<i>Candida albicans</i>	10231	≤10 ³	Good	Good
<i>Enterococcus faecalis</i>	29212	≤10 ³	Good	N/A
<i>Neisseria meningitidis</i>	13090	≤10 ³	Good	Good
<i>Streptococcus pneumoniae</i>	6305	≤10 ³	Good	Good
<i>Streptococcus pyogenes</i>	19615	≤10 ³	Good	Good

