

Liquid media for anaerobic incubation should be reduced prior to inoculation by placing the tubes, with caps loosened, under anaerobic conditions for 18-24 hours prior to use. An efficient and easy way to obtain suitable anaerobic conditions is through the use of BD GasPak™ EZ anaerobic systems or an alternative anaerobic system. Alternatively, liquid media may be reduced immediately prior to use by boiling with caps loosened and cooling with tightened caps to room temperature before inoculation.

Before inoculating Hungate tubes, disinfect the septum of the cap. To inoculate, insert needle of syringe containing specimen through the septum and inject the specimen into the medium. Withdraw the needle slowly to avoid introducing air into the tube.

For use in antimicrobial susceptibility testing, refer to appropriate references.⁸⁻¹⁰

Expected Results

Growth in the tubes is indicated by the presence of turbidity compared to an uninoculated control. If growth appears, cultures should be examined by Gram stain and subcultured onto appropriate media; e.g., a Trypticase™ Soy Agar with 5% Sheep Blood and/or Chocolate II Agar plate, EMB Agar or MacConkey II Agar plate. If anaerobes are suspected, subcultures should be incubated anaerobically, as in a GasPak EZ anaerobic system.

Enterococci will grow in the 6.5% NaCl broth within 24-48 hours. Nonenterococcal group D streptococci fail to grow in the medium after 48 hours of incubation.³

References

1. Rosenow. 1919. J. Dent. Res. 1:205.
2. American Pharmaceutical Association. 1950. The national formulary, 9th ed., APA, Washington, D.C.
3. Pratt-Rippin and Pezzlo. 1992. In Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
4. Barry. 1976. The antimicrobial susceptibility test: principles and practices. Lea & Febiger, Philadelphia, Pa.
5. Fildes. 1920. Br. J. Exp. Pathol. 1:129.
6. Fildes. 1921. Br. J. Exp. Pathol. 2:16.
7. Hungate. 1969. Methods in microbiology. Academic Press, New York, N.Y.
8. Murray, Baron, Jorgensen, Landry and Pfaller, (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

9. Clinical and Laboratory Standards Institute. 2006. Approved Standard: M7-A7, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed. CLSI, Wayne, Pa.

10. Clinical and Laboratory Standards Institute. 2006. Approved Standard M2-A9, Performance standards for antimicrobial disk susceptibility tests, 9th ed. CLSI, Wayne, Pa.

Availability

Bacto™ Brain Heart Infusion

AOAC BAM CCAM CLSI CMPH2 COMPF EPA ISO MCM9
SMD SMWW USDA

Cat. No.	237400	Dehydrated – 100 g
	237500	Dehydrated – 500 g
	237200	Dehydrated – 2 kg
	237300	Dehydrated – 1 kg

BBL™ Brain Heart Infusion

AOAC BAM CCAM CLSI CMPH2 COMPF EPA ISO MCM9
SMD SMWW USDA

Cat. No.	211059	Dehydrated – 500 g
	211060	Dehydrated – 5 lb (2.3 kg)
	211061	Dehydrated – 25 lb (11.3 kg)
	221778	Prepared Tubes, 0.5 mL (K Tubes) – Ctn. of 100
	297769	Prepared Tubes, 2 mL (K Tubes) – Ctn. of 100
	221812	Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10
	221813	Prepared Tubes, 5 mL (K Tubes) – Ctn. of 100
	220837	Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100
	296299	Prepared Bottles, 400 mL – 1 bottle
	297304	Prepared Tubes, Pre-reduced (with Hungate Cap) – Pkg. of 10

BBL™ Brain Heart Infusion with 6.5% Sodium Chloride

Cat. No.	221785	Prepared Tubes – Pkg. of 10
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BBL™ Brain Heart Infusion with 0.1% Agar

Cat. No.	297640	Prepared Tubes, 10 mL (D Tubes) – Ctn. of 100
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BBL™ Brain Heart Infusion with Fildes Enrichment

Cat. No.	297782	Prepared Tubes, 5 mL (K Tubes) – Ctn. of 100*
	297200	Prepared Tubes, 9 mL (K Tubes) – Pkg. of 10*

Difco™ Brain Heart Infusion without Dextrose

Cat. No.	250220	Dehydrated – 10 kg
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BBL™ Brain Heart Infusion Broth, Modified

Cat. No.	299070	Dehydrated – 500 g
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BBL™ Fildes Enrichment

Cat. No.	211866	Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10*
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*Store at 2-8°C.

Brain Heart Infusion Agars

Brain Heart Infusion Agar • Brain Heart Infusion Sheep Blood Agar • Brain Heart Infusion Agar, Modified

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 5% or 10% sheep blood, it is used for the isolation and cultivation of a wide variety of fungal species, including systemic fungi,¹ from clinical and nonclinical sources.

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 that 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.

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

Difco™ Brain Heart Infusion Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in purified water upon boiling. Solution is light to medium amber, slightly opalescent to opalescent with a flocculent precipitate.
Prepared Appearance:	Light to medium amber, slightly opalescent to opalescent with a flocculent precipitate.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Difco™ Brain Heart Infusion Agar

Prepare the medium per label directions without (plain) and with 5% defibrinated sheep blood (SB). Inoculate and incubate at 35 ± 2°C with 5-10% CO₂ for 18-48 hours (incubate *A. brasiliensis* aerobically at 30 ± 2°C for 18-72 hours).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
<i>Aspergillus brasiliensis</i> (niger)	16404	10 ² -3 × 10 ²	Good	Good
<i>Escherichia coli</i>	25922	10 ² -3 × 10 ²	Good	Good
<i>Staphylococcus aureus</i>	25923	10 ² -3 × 10 ²	Good	Good
<i>Streptococcus pneumoniae</i>	6305	10 ² -3 × 10 ²	Good	Good
<i>Streptococcus pyogenes</i>	19615	10 ² -3 × 10 ²	Good	Good

BHI 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. Peptones are also included as sources of nutrients.

Brain Heart Infusion Agar, Modified, the agar form of Brain Heart Infusion, Modified, differs from other formulations by the quantities of the infusion and peptone components utilized.

BHI Agar has proven to be effective in the cultivation of a wide variety of microorganisms, including many types of pathogens. BHI Agar can be used as a general medium for aerobic bacteriology and for the primary recovery of fungi from clinical specimens.² Brain Heart Infusion Agar with 10% Sheep Blood can be used to isolate systemic fungi that may grow poorly on the nonenriched medium. Antimicrobial agents, including chloramphenicol, gentamicin, and penicillin in combination with streptomycin, can be incorporated to improve the recovery of pathogenic fungi from specimens heavily contaminated with bacteria (see Selective Brain Heart Infusion Agars).³

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 a carbohydrate source that

Identity Specifications

BBL™ Brain Heart Infusion Agar

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

BBL™ Brain Heart Infusion Agar, Modified

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	5.3% solution, soluble in purified water upon boiling. Solution is medium to dark, yellow to tan, trace to moderately hazy.
Prepared Appearance:	Medium to dark, yellow to tan, trace to moderately hazy.
Reaction of 5.3% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

BBL™ Brain Heart Infusion Agar

Prepare the medium per label directions without (plain) and with 5% defibrinated sheep blood (SB). Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for 48 hours (incubate *S. rimosus* at 23-27°C for up to 7 days if necessary).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
<i>Escherichia coli</i>	25922	10 ³ -10 ⁴	N/A	Good
<i>Listeria monocytogenes</i>	19115	10 ³ -10 ⁴	N/A	Good
<i>Pseudomonas aeruginosa</i>	10145	10 ³ -10 ⁴	Good	N/A
<i>Shigella flexneri</i>	12022	10 ³ -10 ⁴	Good	N/A
<i>Staphylococcus aureus</i>	25923	10 ³ -10 ⁴	Good	Good
<i>Streptococcus pneumoniae</i>	6305	10 ³ -10 ⁴	Good	Good
<i>Streptococcus pyogenes</i>	19615	10 ³ -10 ⁴	Good	Good
<i>Streptococcus rimosus</i>	10970	Undiluted	Good	N/A

BBL™ Brain Heart Infusion Agar, Modified

Prepare the medium per label directions without (plain) and with 5% defibrinated sheep blood (SB). Inoculate using pour plates and incubate at 35 ± 2°C for 48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
<i>Escherichia coli</i>	25922	10 ³ -10 ⁴	N/A	Good
<i>Staphylococcus aureus</i>	25923	10 ³ -10 ⁴	Good	Good
<i>Streptococcus pyogenes</i>	19615	10 ³ -10 ⁴	Good	Good

microorganisms utilize by fermentative action. The medium is buffered through the use of disodium phosphate.

When defibrinated sheep blood is added to the basal medium, it provides essential growth factors for the more fastidious fungal organisms.

Formulae

Difco™ Brain Heart Infusion Agar

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
Agar	15.0 g

BBL™ Brain Heart Infusion Agar

Approximate Formula* Per Liter	
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

BBL™ Brain Heart Infusion Agar, Modified

Approximate Formula* Per Liter	
Brain Heart, Infusion from (solids)	3.5 g
Peptic Digest of Animal Tissue	15.0 g
Pancreatic Digest of Casein	10.0 g
Dextrose	2.0 g
Sodium Chloride	5.0 g
Disodium Phosphate	2.5 g
Agar	15.0 g

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

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
 Difco™ Brain Heart Infusion Agar – 52 g;
 BBL™ Brain Heart Infusion Agar – 52 g;
 BBL™ Brain Heart Infusion Agar, Modified – 53 g.
 Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Before use, agitate gently to distribute the precipitate uniformly throughout the medium.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Prepare plated medium from tubed 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.

Use standard procedures to obtain isolated colonies from specimens. Since many pathogens require carbon dioxide on primary isolation, plates of plain BHI may be incubated in an atmosphere containing approximately 5-10% CO₂. Incubate plates at 35 ± 2°C for 24-48 hours.

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

Expected Results

After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. When culturing for fungi, examine plates for fungal colonies exhibiting typical color and morphology. Biochemical tests and serological procedures should be performed to confirm findings.

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

References

1. Creitz and Puckett. 1954. *Am. J. Clin. Pathol.* 24:1318.
2. Murray, Baron, Jorgensen, Landry and Pfaller, (ed.). 2007. *Manual of clinical microbiology*, 9th ed. American Society for Microbiology, Washington, D.C.
3. Reisner, Woods, Thompson, 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.

Availability

Difco™ Brain Heart Infusion Agar

AOAC BAM CCAM COMPF EPA MCM9 SMD SMWW USDA

Cat. No.	241820	Dehydrated – 100 g
	241830	Dehydrated – 500 g
	241810	Dehydrated – 2 kg

BBL™ Brain Heart Infusion Agar

AOAC BAM CCAM COMPF EPA MCM9 SMD SMWW USDA

Cat. No.	211065	Dehydrated – 500 g
	212166	Dehydrated – 5 lb (2.3 kg)

United States and Canada

Cat. No.	221569	Prepared Plates (Deep Fill) – Pkg. of 20*
	221570	Prepared Plates (Deep Fill) – Ctn. of 100*
	220838	Prepared Pour Tubes (20 mL) – Pkg. of 10
	221610	Prepared Slants (K Tubes) – Pkg. of 10
	297283	Prepared Slants (A Tubes) – Pkg. of 10

Europe

Cat. No.	255003	Prepared Plates – Pkg. of 20*
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Japan

Cat. No.	252109	Prepared Plates (Deep Fill) – Pkg. of 20*
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BBL™ Brain Heart Infusion Agar with 5% Sheep Blood

BS12 CMPH2 MCM9

Cat. No.	297199	Prepared Slants – Pkg. of 10*
	296067	Prepared Slants – Ctn. of 100*

BBL™ Brain Heart Infusion Agar with 10% Sheep Blood

BS12 CMPH2 MCM9

United States and Canada

Cat. No.	296125	Prepared Slants – Pkg. of 10*
	221843	Prepared Plates (Deep Fill) – Pkg. of 10*

BBL™ Brain Heart Infusion Agar, Modified

Cat. No.	299069	Dehydrated – 500 g
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*Store at 2-8°C.