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 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

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
Neisseria meningitidis	13090	10^2-10^3	Good
Streptococcus pneumoniae	6305	10^2-10^3	Good
Streptococcus pyogenes	19615	10^2-10^3	Good

BBL™ Brain Heart Infusion Broth, 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

**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:
   Bacto™ Brain Heart Infusion – 37 g;
   BBL™ Brain Heart Infusion – 37 g;
   Difco™ Brain Heart Infusion without Dextrose – 35 g;
   BBL™ Brain Heart Infusion Broth, Modified – 38 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. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

With liquid specimens, tubed media should be inoculated with 1-2 drops of the specimen using a sterile pipette. Swab specimens may be inserted into broth after inoculation of plated media.

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

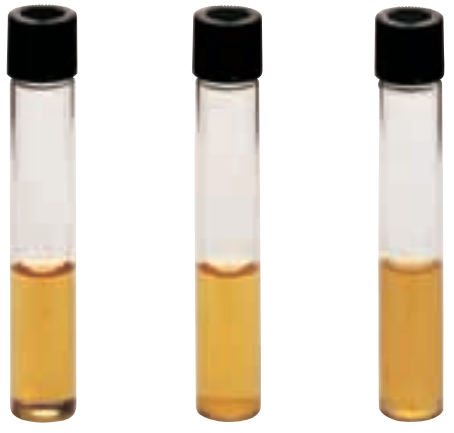
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.8% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 3.7% Solution 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
Bacteroides fragilis	25285	≤10^4	Good	Good
Candida albicans	10231	≤10^3	Good	Good
Enterococcus faecalis	29212	≤10^3	Good	N/A
Neisseria meningitidis	13090	≤10^3	Good	Good
Streptococcus pneumoniae	6305	≤10^3	Good	Good
Streptococcus pyogenes	19615	≤10^3	Good	Good



Uninoculated Tube | *Neisseria meningitidis* ATCC™ 13090 | *Streptococcus pyogenes* ATCC™ 19615

Brain Heart Infusion

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 antimicrobic 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

BBL™ Brain Heart Infusion with 0.1% Agar

Cat. No.	297640	Prepared Tubes, 10 mL (D Tubes) – Ctn. of 100

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

BBL™ Brain Heart Infusion Broth, Modified

Cat. No.	299070	Dehydrated – 500 g

BBL™ Fildes Enrichment

Cat. No.	211866	Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10*

**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.