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.

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 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.

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% CO_2 for 18-48 hours (incubate *A. niger* aerobically at 30 ± 2°C for 18-72 hours).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
Aspergillus niger	16404	10^2-3×10^2	Good	Good
Escherichia coli	25922	10^2-3×10^2	Good	Good
Staphylococcus aureus	25923	10^2-3×10^2	Good	Good
Streptococcus pneumoniae	6305	10^2-3×10^2	Good	Good
Streptococcus pyogenes	19615	10^2-3×10^2	Good	Good

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% CO_2. 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.

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
Escherichia coli	25922	10^3-10^4	N/A	Good
Listeria monocytogenes	19115	10^3-10^4	N/A	Good
Pseudomonas aeruginosa	10145	10^3-10^4	Good	N/A
Shigella flexneri	12022	10^3-10^4	Good	N/A
Staphylococcus aureus	25923	10^3-10^4	Good	Good
Streptococcus pneumoniae	6305	10^3-10^4	Good	Good
Streptococcus pyogenes	19615	10^3-10^4	Good	Good
Streptococcus rimosus	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
Escherichia coli	25922	10^3-10^4	N/A	Good
Staphylococcus aureus	25923	10^3-10^4	Good	Good
Streptococcus pyogenes	19615	10^3-10^4	Good	Good

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. Chapin and Murray. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Reisner, Woods, Thompson, Larone, Garcia and Shimizu. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Brain Heart Infusion Agar

AOAC | BAM | BS10 | CMPH | COMPF | MCM7 | SMD | SMWW | USDA

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

BBL™ Brain Heart Infusion Agar

AOAC | BAM | BS10 | CMPH | COMPF | MCM7 | SMD | SMWW | USDA

Cat. No.	211065	Dehydrated – 500 g
	212166	Dehydrated – 5 lb (2.3 kg)
	212247	Dehydrated – 25 lb (11.3 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*

BBL™ Brain Heart Infusion Agar with 5% Sheep Blood

BS10 | CMPH | MCM7

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

BBL™ Brain Heart Infusion Agar with 10% Sheep Blood

BS10 | CMPH | MCM7

United States and Canada

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

Europe

Cat. No.	255544	Prepared Plates – Pkg. of 20*

BBL™ Brain Heart Infusion Agar, Modified

Cat. No.	299069	Dehydrated – 500 g

**Store at 2-8°C.*

Bacto™ Brain Heart Infusion, Porcine

Intended Use

Bacto™ Brain Heart Infusion, Porcine is used for cultivating a wide variety of microorganisms.

Summary and Explanation

Rosenow[1] devised an excellent medium for culturing streptococci by supplementing Dextrose Broth with brain tissue. Hayden,[2] revising Rosenow's procedure by adding crushed marble to the medium, reported favorable growth of organisms from dental pathogens. Brain Heart Infusion is a modification of the media described by Rosenow[1] and Hayden.[2] Infusion from calf brains has replaced the brain tissue and disodium phosphate has replaced the calcium carbonate buffer.

Brain Heart Infusion, Porcine was developed as an alternative to the Brain Heart Infusion (BHI) formula, and replaces calf brains and beef heart with porcine brains and heart. Brain Heart Infusion, Porcine was developed for pharmaceutical and vaccine production and can replace the traditional BHI depending on organism and production application. BHI, Porcine was formulated with no bovine components to minimize Bovine Spongiform Encephalopathy (BSE) risk.

The nutritionally rich formula of BHI is used to grow a variety of microorganisms. The original Brain Heart Infusion media are specified in standard methods for multiple applications.[3-6]

User Quality Control

Identity Specifications

Bacto™ Brain Heart Infusion, Porcine

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in purified water. 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

Cultural Response

Bacto™ Brain Heart Infusion, Porcine

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	Fair
Streptococcus pneumoniae	6305	10^2-10^3	Good
Streptococcus pyogenes	19615	10^2-10^3	Fair

Principles of the Procedure

Infusion from pork brains, infusion from pork heart and Pork Peptone No. 2 provide nitrogen, carbon, sulfur and vitamins in Brain Heart Infusion, Porcine. Dextrose is the carbon energy source to facilitate organism growth. Sodium chloride maintains the osmotic balance of the medium. Disodium phosphate is the buffering agent.