BRAIN HEART INFUSION AGAR (BHIA)
MYCOLOGY

PRODUCTS:
Plated, Tubed, and Bottled Media:

Brain Heart Infusion Agar, Plain	P1150, P1152, T6220 (10ml), T6222 (5ml), B5403
Brain Heart Infusion Agar with Cyclohexamide and Chloramphenicol	P1153
Brain Heart Infusion Agar + 5% Sheep Blood	P1160, P1162, T6225
Brain Heart Infusion Agar + 6% Sheep Blood and Chloramphenicol	T6227
Brain Heart Infusion Agar + 5% Sheep Blood with Chloramphenicol and Cyclohexamide	T6235
Brain Heart Infusion Agar + 5% Sheep Blood with Chloramphenicol, Gentamicin, and Cyclohexamide	B5406
Brain Heart Infusion Agar + 5% Sheep Blood with Chloramphenicol and Gentamicin	T6229, T6230
Brain Heart Infusion Agar + 10% Sheep Blood	P1165
Brain Heart Infusion Agar + 10% Sheep Blood with Chloramphenicol and Gentamicin	P1163
Brain Heart Infusion Agar + 10% Sheep Blood with with Chloramphenicol, Gentamicin, and Cyclohexamide	P1164

PURPOSE:
Brain Heart Infusion Agar (BHIA) is a nutritious base media used for the cultivation of a wide variety of organism types, including bacteria, yeasts, and molds. With the addition of 5-10% sheep blood, it is used for the cultivation and isolation of fastidious dimorphic fungi, e.g., *Blastomyces dermatitidis, Coccidioides immitis, Cryptococcus neoformans, Histoplasma capsulatum, Paracoccidioides brasiliensis*, and *Sporothrix schenckii*. BHIA is used in combination with other mycology media to ensure recovery of all clinically significant fungi.

PRINCIPLE:
Brain Heart Infusion Broth was used by Rosenow[9] and other investigators because it proved effective in the cultivation of a variety of microbes, e.g., streptococci and dental pathogens, from infected tissue.[2,4,5,9] Over time the formula was modified by the addition of agar, by the substitution of infusion of calf brain for the original brain tissue, and by the addition of a variety of supplements and enrichments which further enhanced the recovery of microorganisms. Enriched BHIA media also proved effective in the recovery of fastidious fungi; the addition of 5-10% sheep blood has further ensured the recovery of dimorphic fungi. The predominant sites from which dimorphic fungi have been recovered are the lower respiratory tract and skin;[8] both sites harbor other microbes necessitating the addition of antimicrobial agents to BHIA to inhibit the normal flora and to allow the cultivation of the dimorphic, slower-growing fungi. Traditionally, 400-500 mcg/ml of cycloheximide has been added as the antifungal agent and 50 mcg/ml of chloramphenicol served as the antibacterial agent. Recently, the combination of 5 mcg/ml of gentamicin and 16 mcg/ml of chloramphenicol has proven more effective in inhibiting bacteria.[1,9]

No single culture media effectively recovers all fungal etiologic agents; the use of a battery of media is required. Sterile sites, such as cerebrospinal fluid (CSF) and blood, require enriched media, which allows clinically significant fungi to grow and produce spores. Other sites may have normal bacterial and fungal flora, which may interfere with the growth of clinically significant fungi and, in addition, the cultures may require incubation for a month or more; all of which necessitate the use of media with antimicrobial agents. Each institution needs to establish the combination or battery of media that allows the optimal recovery of clinically significant fungi and which method to use. Plated media provide better aeration of the culture, greater surface area for better isolation, and are optimal for the recovery of fungi.[3,8] However, inadvertent opening during handling and incubation necessitates taping the plate shut. Due to the extended incubation period, plates must have at least 40 ml of agar and the humidity in the incubator needs to be 40-50%. Screw-cap tubes are safer to handle, easier to store, and have a lower dehydration rate. However, the surface area for growth is small and the fungi may concentrate near the bottom of the tube. Alleviation of the above-mentioned tube limitations requires the use of larger tubes and incubating the tubes in a horizontal position for the first 24 hours.

FORMULAS:

Approximate, per liter deionized filtered water.

(1) Brain Heart Infusion Agar, Plain:
Calf Brain-Beef Heart Infusion Solids.....17.5 g
Peptic Digest of Animal Tissue..... 5.0
Pancreatic Digest of Casein..... 5.0
Dextrose..... 2.0
Sodium Chloride..... 5.0
Dibasic Sodium Phosphate..... 2.5
Agar.....15.0
Final pH 7.4 ± 0.2 at 25°C

(2) Brain Heart Infusion Agar with Chloramphenicol and Cyclohexamide:
Same as **(1)** with the addition of 50.0 mg Choramphenicol and 0.5 mg of Cyclohexamide

(3) Brain Heart Infusion Agar + 5% Sheep Blood:
Same as **(1)** with the addition 50.0 ml of Sheep Blood

(4) Brain Heart Infusion Agar + 6% Sheep Blood and Chloramphenicol:
Same as **(3)** with the addition 50.0 mg of Chloramphenicol and 10.0 ml Sheep Blood

(5) Brain Heart Infusion Agar + 5% Sheep Blood, Chloramphenicol and Cycloheximide:
Same as **(3)** with 50.0 mg of Chloramphenicol and 0.5 g of Cycloheximide

(6) Brain Heart Infusion Agar + 5% Sheep Blood, Chloramphenicol, Gentamicin, and Cycloheximide:
Same as **(3)** with 50.0 mg of Chloramphenicol, 8.0 mg Gentamicin, and 0.5 g of Cycloheximide

(7) Brain Heart Infusion Agar + 5% Sheep Blood, Chloramphenicol and Gentamicin:
Same as **(3)** with 50.0 mg of Chloramphenicol and 8.0 mg Gentamicin

(8) Brain Heart Infusion Agar + 10% Sheep Blood:
Same as **(1)** with the addition 100.0 ml of Sheep Blood

(9) Brain Heart Infusion Agar With 10% Sheep Blood, Chloramphenicol, and Gentamicin:
Same as **(8)** with the addition of 16.0 mg of Chloramphenicol and 5.0 mg of Gentamicin

(10) Brain Heart Infusion Agar With 10 % Sheep Blood, Chloramphenicol, Gentamicin, and Cycloheximide:
Same as **(8)** with16.0 mg of Chloramphenicol, 5.0 mg of Gentamicin, and 0.5 g of Cycloheximide

PRECAUTIONS:*

For *in vitro* diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (cracking, shrinking, or discoloration), or if the expiration date has passed.

Limitations: If BHIA with sheep blood is used, subculturing to a less enriched media, such as Sabouraud Dextrose Agar or Potato Dextrose Agar, may be necessary to induce sporulation and the delineation of identifiable structures.

The use of screw-cap tubes provides relatively poor aeration of cultures, due to the small surface area, colony isolation is poor.

Use of plated media increases the chance of contamination since the system is not closed. It also increases the rate of media

dehydration.

Excessive concentration of antimicrobials may lead to the inhibition of certain fungi.

Cycloheximide partially or completely inhibits *Cryptococcus neoformans, Aspergillus fumigatus, Candida krusei, Trichosporon beiglii*, and *Pseudallescheria boydii*.

The taping of plates may be necessary to reduce dehydration and reduce the risk of aerial dissemination of spores.

This media is not recommended for determining hemolytic reactions due to the high dextrose content.

PROCEDURE:*

Specimen Collection: Information on specimen collection is found in standard reference material. In general, specimens from normally sterile sites should be collected with good skin antisepsis, specimens should be protected from extreme heat and cold, and delivered to the laboratory without delay.

Method of Use: Prior to inoculation, the media should be brought to room temperature. Cutaneous specimens, biopsy, or autopsy specimens should be lightly embedded in the agar or, if large, macerated in sterile saline in a tissue grinder and the sediment inoculated onto the plates. Specimens from swabs or liquid specimens should be lightly streaked across the agar surface, using a fishtail motion. BHIA media should be held aerobically at 25-30°C, with caps loose, and examined at regular intervals for 4-6 weeks. If growth occurs, subculture to appropriate media and perform appropriate macroscopic, microscopic, and biochemical tests to secure a definitive identification of the organism. Consult appropriate reference texts for details of identification.[3,6,8,]

Interpretation: Once growth occurs, note gross appearance (topography, texture, and pigmentation), temperature at which growth occurs, and rate of growth. Subculture to appropriate media and perform specific microscopic and biochemical tests to reach a definitive identification.

Materials Required but Not Provided: Standard microbiological supplies and equipment, as well as other less-enriched media for subculturing, and other materials necessary for identification are not provided.

QUALITY CONTROL:*

Media Used:	Microorganisms Used (ATCC#):	Expected Results:
BHIA, Plain	*Candida albicans* (10231)	Growth
	Escherichia coli (25922)	Growth
	Haemophilus influenzae (10211) [plated media only]	Growth with XV disk
	Trichophyton mentagrophytes (9533) [double pour plates]	Growth
BHIA with Cyclohexamide and Chloramphenicol	*Candida albicans* (10231)	Growth
	Candida albicans (60193)	Growth
	Trichophyton mentagrophytes (9533)	Growth
	Candida krusei (14243)	No growth
	Escherichia coli (25922)	Inhibition, partial to complete
BHIA + Sheep Blood	*Streptococcus pyogenes* (19615)	Growth, beta hemolysis
	Candida albicans (10231)	Growth
BHIA + 6% Sheep Blood and Chloramphenicol	*Candida albicans* (10231)	Growth
	Aspergillus brasiliensis (16404)	Growth
	Trichophyton mentagrophytes (9533)	Growth
	Escherichia coli (25922)	Inhibition, partial to complete
BHIA + Sheep Blood and Antibiotics	*Candida albicans* (10231)	Growth
	Candida krusei (14243)	No growth in presence of cycloheximide; Growth in presence of gentamicin, chloramphenicol
	Escherichia coli (25922)	Inhibition, partial to complete

User Quality Control: Check for signs of contamination and deterioration. Brain Heart Infusion Agar should appear firm, translucent, and light yellow in color. Brain Heart Infusion Agar with blood should appear firm, opaque, and red in color.

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* For more detailed information, consult appropriate references.

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