BRAIN HEART INFUSION (BHI) MEDIA

PRODUCT:

Plate, Tube, and Bottle Media:[a]

Brain Heart Infusion Broth*	T6241,T3070,T6243,T6240, T3075,T6242
Brain Heart Infusion Broth With Enrichment(s)	T6252, T6250 (1%XV), T6239 (fildes)
Brain Heart Infusion Agar	P1150
Brain Heart Infusion Agar with PABA	T6238

[a]Available in several volumes and tube sizes, consult catalog, website, or call PML for ordering options
SEE MYCOLOGY SECTION FOR ADDITIONAL BHI MEDIA (DATA #190)

PURPOSE:

Brain heart infusion is a nutrient medium used for the isolation and cultivation of a variety of microorganisms. BHIA can be used as a nonblood-containing medium to identify X and V factor requirements of *Haemophilus* species. Fildes-enriched agar is useful for demonstrating capsular strains of *Haemophilus influenzae*.

PRINCIPLE:

Brain heart infusion was first used by Rosenow,[10] who added brain tissue to dextrose broth, and discovered a medium that proved useful in the cultivation of streptococci. Other investigators modified the formula and discovered it effective in the recovery of microorganisms from infected tissue and in the recovery of dental pathogens.[2,5,7] The standard formula now consists of infusion of calf brain, which is a clearer medium with equivalent nutritive value. In addition, a variety of supplements and enrichments have been added to enhance further the recovery of fastidious microorganisms.

Fildes, a peptic digest of sheep blood which is rich in X and V factors, is added to enhance the recovery of *Haemophilus* species and other microorganisms requiring blood derivatives.[3,4]

XV Factor Enrichment is a chemically defined enrichment that replaces the conventional yeast extract supplement and contains additional cofactors, vitamins, and amino acids required by many fastidious microorganisms.

Hemin and Vitamin K_1 are enrichments used to enhance the cultivation of anaerobes.

A small amount of agar added to a broth creates a condition of a varying amount of oxygen tension and provides an optimum condition for the cultivation of aerobes, microaerophiles and obligate anaerobes.

PABA is used to neutralize the bacteriostatic effects of sulfa drugs and to enhance the growth of pathogenic organisms.

FORMULAS:

Approximate, per liter deionized filtered water.

(1) Brain Heart Infusion (BHI) Broth:

Calf Brain-Beef Heart Infusion (Solids)	17.5 gm
Pancreatic Digest of Gelatin	10.0
Dextrose	2.0
Sodium Chloride	5.0
Disodium Phosphate	2.5

Final pH 7.4 ± 0.2 at 25°C

(2) BHI With Fildes Enrichment:
Same as (1) with 50.0 ml of Fildes Enrichment.

(3) BHI With XV Factor Enrichment:
Same as (1) with 10.0 ml of XV Factor Enrichment.

(4) BHI With PABA:
Same as (1) with 50.0 mg of Para-Aminobenzoic Acid and 1.0 g of Agar.

(5) Brain Heart Infusion Agar (BHIA):

Calf Brain-Beef Heart Infusion (Solids)	17.5 gm
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

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 (shrinking, cracking, evaporation or discoloration), or if the expiration date has passed.

Limitations: Brain heart infusion media serve as nonselective media; biochemical and/or serologic testing are necessary for definitive identification of microorganisms.

Many X factor-requiring organisms can carry over X factor from primary culture media. Thus, the X and V factor disk method utilizing nonblood-containing media may misidentify *Haemophilus influenzae*.[9] There is no complex medium which will otherwise support the growth of *Haemophilus* species that is totally free of X factor. Even when care is taken to avoid carryover with inoculum, the XV strip/disk method has an error level of approximately 18 percent.[9]

PROCEDURES: *

Specimen Collection: Information on specimen collection is found in standard reference material. In general, specimens should be protected from extremes of heat and cold and should be delivered to the laboratory immediately. If there is a delay, a suitable transport media such as Amies must be used to maintain the viability of the organisms.

Method of Use: Prior to inoculation, the medium should be brought to room temperature. To prepare the plated media from the 18-ml agar deep tube, heat the tube in a boiling water bath until the agar is melted, cool to 50°C, pour the melted medium into a sterile petri dish, and cool to room temperature. Inoculate according to standard microbiological procedures. On plate media, streak inoculum in order to obtain isolated colonies. Incubate under conditions that will permit growth of microbes. In general, incubate at 35°C with adequate moisture in either aerobic, capnophilic, or anaerobic environments, depending on the specific microbes to be cultured.

X, V and XV Disk/Strip Method: Emulsify a 24-hour-old colony in saline; a dilute suspension of the microorganism is used to prevent carryover of X factor. A plate of plain BHIA is inoculated with a swab saturated with the dilute suspension. Place X, V and XV disks/strips according to the manufacturer's instructions. For more detailed instructions and interpretation, consult appropriate references. Incubate in 5-10% CO_2 and examine the disks/strips after 18-24 hours.

Interpretation: Subculture growth seen from primary cultures to appropriate media and perform specific biochemical and/or serological tests in order to reach a definitive identification.

For X, V Disk/Strip Method, growth between the X and V strips indicates a requirement for both factors; growth around a single disk/strip indicates a requirement for the specific factor or factors.[6]

Materials Required but Not Provided: Standard microbiological supplies and equipment, and test strips are not provided.

QUALITY CONTROL:*

Media Used:	Microorganisms Used	ATCC #	Expected Results
BHIA:	*Candida albicans*	10231	Growth
	Escherichia coli	25922	Growth
	Trichophyton mentagrophytes	9533	Growth
BHI With Fildes, XV:			
	Haemophilus influenzae	10211	Growth
	Neisseria meningitidis	13090	Growth
BHI Without Enrichments & BHI With PABA:			
	Escherechia coli	25922	Growth
	Staphylococcus aureus	25923	Growth

User Quality Control: Check for signs of contamination and deterioration.

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* For more detailed information, consult appropriate references.

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