

# Instructions for Use

## BRAIN HEART INFUSION (BHI) AGAR

<a href="#">Cat. no. L35</a>	BHI Agar, 20x125mm Tube, 10ml Slant	20 tubes/box
<a href="#">Cat. no. L36</a>	BHI Agar, 16x100mm Tube, 5.5ml Slant	20 tubes/box
<a href="#">Cat. no. Q16</a>	BHI Agar, 20x150mm Tube, 18ml Deep	20 tubes/box
<a href="#">Cat. no. W15</a>	BHI Agar, 15x100mm Plate, 26ml	10 plates/bag
<a href="#">Cat. no. W163</a>	BHI Agar, 25x100mm Plate, 60ml	5 plates/bag
<a href="#">Cat. no. X10</a>	BHI Agar, 50ml HardyFlask™, 12ml Slant	20 flasks/box
<a href="#">Cat. no. A20</a>	BHI Agar with Blood, 15x100mm Plate, 18ml	10 plates/bag
<a href="#">Cat. no. W185BX</a>	BHI Agar with Blood, 25x100mm Plate, 37ml	50 plates/box
<a href="#">Cat. no. X13</a>	BHI Agar with Blood, 50ml HardyFlask™, 12ml	20 flasks/box
<a href="#">Cat. no. L31</a>	BHI Agar with Blood, 20x125mm Tube, 10ml Slant	20 tubes/box
<a href="#">Cat. no. X12</a>	BHI Agar with Blood and Gentamicin, 50ml HardyFlask™, 12ml	20 flasks/box
<a href="#">Cat. no. L114</a>	BHI Agar with Blood, Chloramphenicol and Gentamicin, 20x125mm Tube, 10ml Slant	20 tubes/box
<a href="#">Cat. no. W65</a>	BHI Agar with Blood, Chloramphenicol and Gentamicin, 15x100mm Plate, 26ml	10 plates/bag
<a href="#">Cat. no. X14</a>	BHI Agar with Blood, Chloramphenicol and Gentamicin, 50ml HardyFlask™, 12ml	20 flasks/box
<a href="#">Cat. no. X11</a>	BHI Agar with Chloramphenicol and Cycloheximide, 50ml HardyFlask™, 12ml	20 flasks/box

## INTENDED USE

Hardy Diagnostics Brain Heart Infusion (BHI) Agar is a general purpose nutrient medium recommended for the cultivation and isolation of a variety of microorganisms, including bacteria, yeasts, and molds. The addition of defibrinated sheep blood and antimicrobics produces a selective medium used for the isolation of pathogenic fungi from specimens heavily contaminated with bacteria and saprophytic fungi.<sup>(2)</sup>

## SUMMARY

Rosenow, by adding brain tissue to dextrose broth, discovered a medium useful in the cultivation of streptococci.<sup>(8)</sup> Formula modifications were made by various researchers who found the medium effective in the recovery of dental pathogens.<sup>(4,5,8)</sup> The medium was further modified by the addition of agar and a variety of supplements and enrichments which further enhanced the recovery of microorganisms.

The brain heart infusion, peptone and dextrose components of the medium provide the nutrients to BHI Agar. Organic nitrogen, carbon, sulfur, vitamins and trace substances are provided by the peptones and infusion. Dextrose provides

the carbohydrate source for fermentative microorganisms. Disodium phosphate is added to the medium in order to maintain an optimal pH. Additionally, a variety of supplements has been added to further enhance the recovery of fastidious microorganisms.

BHI Agar with Blood is used for the recovery of fungal species.<sup>(13)</sup> Blood provides essential growth factors for the more fastidious fungal organisms.

BHI Agar with Blood, Gentamicin and Chloramphenicol is used for the selective isolation of fungi from specimens heavily contaminated with bacteria.<sup>(2)</sup> Gentamicin inhibits the growth of gram-negative and some gram-positive bacteria. A wide range of gram-positive and gram-negative bacteria are inhibited by chloramphenicol, a broad-spectrum antimicrobial.

BHI Agar with Chloramphenicol and Cycloheximide is used for the selective isolation of pathogenic fungi. Cycloheximide inhibits saprophytic mold while Chloramphenicol acts as a broad spectrum antibacterial agent.<sup>(2)</sup>

## FORMULA

Ingredients per liter of deionized water:\*

<b>BHI Agar:</b>	
Pancreatic Digest of Casein	16.0gm
Brain Heart Infusion from Solids	10.0gm
Peptic Digest of Animal Tissue	5.0gm
Sodium Chloride	3.0gm
Disodium Phosphate	2.5gm
Dextrose	2.0gm
Agar	13.5gm
<b>BHI Agar with Blood:</b>	
<i>In addition to the above formulation, contains:</i>	
Sheep Blood	50.0ml
<b>BHI Agar with Blood and Gentamicin:</b>	
<i>In addition to the above formulation, contains:</i>	
Gentamicin	5.0mg
Sheep Blood	50.0ml
<b>BHI Agar with Blood, Chloramphenicol and Gentamicin**:</b>	
<i>In addition to the above formulation, contains:</i>	
Chloramphenicol	50.0mg
Gentamicin	5.0mg
Sheep Blood	50.0ml
<b>BHI Agar with Chloramphenicol and Cycloheximide**:</b>	
Pancreatic Digest of Casein	16.0gm
Brain Heart Infusion	8.0gm

Peptic Digest of Animal Tissue	5.0gm
Sodium Chloride	5.0gm
Disodium Phosphate	2.5gm
Dextrose	2.0gm
Cycloheximide	0.5gm
Chloramphenicol	0.05gm
Agar	13.5gm

Final pH 7.4 +/- 0.3 at 25°C.

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

\*\* This media contains chloramphenicol and/or cycloheximide which are ingredients considered potentially hazardous.

## STORAGE AND SHELF LIFE

Storage: Upon receipt store all blood and antibiotic containing BHI Agar products\* at 2-8°C (**\*except** Cat. nos. L35, X10 and X11). Cat. nos. L35, X10 and X11 may be stored at 2-30°C. Products should not be used if there are any signs of contamination, deterioration (shrinking, cracking, or discoloration), hemolysis, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

## PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

## PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.<sup>(1-3,6)</sup> Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport medium and refrigerated until

inoculation.

Method of Use for Cultivation and Isolation of Bacteria: Consult listed references for the appropriate cultivation techniques using this medium.<sup>(1-3,6)</sup> Medium should be brought to room temperature and agar surface should be dry prior to inoculation. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface. Streak for isolation with a sterile loop. Incubate plates aerobically at 35 +/- 2.0°C for 24 to 48 hours. Protect from light. Examine plates for colonial morphology.

Method of Use for Cultivation and Isolation of Fungi: A selective and non-selective medium should be inoculated for isolation of fungi from potentially contaminated specimens. Media should be incubated at room temperature (15-30°C) with increased humidity. Two sets of media should be inoculated for isolation of fungi causing systemic mycoses; one set incubated at 15-30°C and one set at 35 +/- 2.0°C. Cultures should be examined weekly for fungal growth. Media should be held for four to six weeks before being reported as negative.

BHI Agar slants (without supplements or antimicrobics) are used primarily for the cultivation and maintenance of pure cultures of microorganisms.

## INTERPRETATION OF RESULTS

Media should be examined for typical bacterial and fungal colonial morphology. Consult listed references for the interpretation of growth and other identification tests to identify growth of organism(s) on this media.<sup>(1-3,6)</sup>

## LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

## MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as inoculating loops, other culture media, swabs, applicator sticks, incinerators, refrigerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

## QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<b>BHI Agar:</b>					
<i>Escherichia coli</i> ATCC® 25922	A	24-48hr	35°C	Aerobic	Growth
<i>Staphylococcus aureus</i> ATCC® 25923	A	24-48hr	35°C	Aerobic	Growth
<i>Staphylococcus epidermidis</i> ATCC® 12228	A	18-24hr	35°C	Aerobic	Growth
<b>BHI Agar with Blood:</b>					

<i>Streptococcus pyogenes</i> ATCC® 19615	A	18-24hr	35°C	Aerobic	Growth; beta-hemolysis
<i>Streptococcus pneumoniae</i> ATCC® 6305**	A	18-24hr	35°C	Aerobic	Growth; alpha-hemolysis
<i>Aspergillus brasiliensis</i> ATCC® 16404	G	5-7 days	30°C	Aerobic	Growth
<i>Staphylococcus aureus</i> ATCC® 25923	A	18-24hr	35°C	Aerobic	Growth
<i>Escherichia coli</i> ATCC® 25922	A	18-24hr	35°C	Aerobic	Growth
<b>BHI Agar with Blood and Gentamicin / BHI Agar with Blood, Chloramphenicol, and Gentamicin:</b>					
<i>Candida albicans</i> ATCC® 10231**	A	18-24hr	35°C	Aerobic	Growth
<i>Trichophyton interdigitale</i> ATCC® 9533	G	7 days	15-30°C	Aerobic	Growth
<i>Escherichia coli</i> ATCC® 25922	B	18-24hr	35°C	Aerobic	Partial to complete inhibition
<i>Pseudomonas aeruginosa</i> ATCC® 27853	B	24hr	35°C	Aerobic	Partial to complete inhibition
<b>BHI Agar with Chloramphenicol and Cycloheximide:</b>					
<i>Candida albicans</i> ATCC® 10231**	A	18-24hr	35°C	Aerobic	Growth
<i>Trichophyton interdigitale</i> ATCC® 9533**	G	7 days	15-30°C	Aerobic	Growth
<i>Escherichia coli</i> ATCC® 25922**	B	18-24hr	35°C	Aerobic	Partial to complete inhibition
<i>Aspergillus brasiliensis</i> ATCC® 16404**	G	7 days	15-30°C	Aerobic	Partial to complete inhibition

\* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

\*\* Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

## USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

## PHYSICAL APPEARANCE

BHI Agar and BHI Agar with Chloramphenicol and Cycloheximide should appear slightly opalescent, and light amber in color.

BHI Agar with Blood; BHI Agar with Blood and Gentamicin; and BHI Agar with Blood, Chloramphenicol, and Gentamicin should appear opaque, and cherry to brick-red in color.



Sterile plate of BHI Agar (Cat. no. W15).



*Escherichia coli* (ATCC® 25922) colonies growing on BHI Agar (Cat. no. W15). Incubated aerobically for 24 hours at 35°C.



Uninoculated plate of BHI Agar with Blood (Cat. no. A20).



*Candida albicans* (ATCC® 10231) colonies growing on BHI Agar with Blood, Chloramphenicol and Gentamicin (Cat. no. W65). Incubated aerobically for 24 hours at 35°C.



*Streptococcus pneumoniae* (ATCC® 6305) colonies growing on BHI Agar with Blood (Cat. no. A20). Incubated aerobically for 24



*Escherichia coli* (ATCC® 25922) growth inhibited on BHI Agar with Blood, Chloramphenicol and Gentamicin (Cat. no. W65).

hours at 35°C.

Incubated aerobically for 24 hours at 35°C.



*Trichophyton interdigitale* (ATCC® 9533) colonies growing on BHI Agar with Blood, Chloramphenicol and Gentamicin (Cat. no. W65). Incubated aerobically for 96 hours at 30°C.

## REFERENCES

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ATCC is a registered trademark of the American Type Culture Collection.

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