BRAIN HEART INFUSION (BHI) AGAR w/ and w/o ADDITIVES

INTENDED USE
Remel Brain Heart Infusion Agar w/ and w/o Additives are solid media recommended for use in the cultivation of a wide variety of microorganisms including pathogenic fungi.

SUMMARY AND EXPLANATION
Brain Heart Infusion (BHI) Agar is a nutritious base medium suitable for the cultivation of a wide variety of microorganisms including bacteria, yeasts, and molds.[1] With the addition of 5-10% sheep blood, BHI Agar can be used for the isolation of dimorphic fungi which may grow poorly on nonenriched media. Rosebury et al. tested the suitability of BHI Agar to support the growth of *Actinomyces*.[2] Dolan determined optimal concentrations of chloramphenicol for the isolation of pathogenic fungi and *Nocardia asteroides*.[3]

PRINCIPLE
This medium contains brain heart infusion, peptone, and dextrose which provide nitrogen, carbon, sulfur, vitamins, and a carbohydrate source. Sheep blood provides essential growth factors for fastidious fungi. Yeast extract may be incorporated in the medium to serve as a growth stimulant. Gentamicin is an aminoglycoside which inhibits some gram-negative bacteria. Chloramphenicol is a broad-spectrum antibiotic which inhibits a range of gram-positive and gram-negative organisms. Penicillin is active against gram-positive bacteria and streptomycin inhibits a variety of gram-positive and gram-negative bacteria. Cycloheximide inhibits rapidly growing saprophytic fungi that may overgrow slower-growing pathogens; however, it also inhibits the growth of some significant pathogens (e.g., *Cryptococcus neoformans*, some *Candida* spp., some *Aspergillus* spp., and zygomycetes).

REAGENTS (CLASSICAL FORMULA)*
Base Medium:

Casein Peptone	14.5 g	Disodium Phosphate	2.5 g
Meat Peptone	7.0 g	Dextrose	2.0 g
Brain Heart Infusion	6.0 g	Sheep Blood (optional)	6 %
Sodium Chloride	5.0 g	Agar	15.0 g
		Demineralized Water	1000.0 ml

pH 7.4 ± 0.2 @ 25°C

The following combinations of optional ingredients are available per liter of medium:

1. Chloramphenicol	50.0 mg	5. Penicillin	20,000 U
2. Chloramphenicol	50.0 mg	Streptomycin	40.0 g
Gentamicin	40.0 mg	6. Penicillin	20,000 U
3. Chloramphenicol	50.0 mg	Streptomycin	40.0 mg
Gentamicin	40.0 mg	Cycloheximide	500.0 g
Cycloheximide	500.0 mg	7. Penicillin	20,000 U
4. Chloramphenicol	50.0 mg	Streptomycin	40.0 mg
Cycloheximide	500.0 mg	Yeast Extract	10.0 mg

*Adjusted as required to meet performance standards.

PROCEDURE
1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory. A selective and nonselective medium should be inoculated for isolation of fungi from potentially contaminated specimens.
2. Incubate the plates aerobically at 25-30°C in an inverted position with increased humidity for 30 days or longer.
3. Examine plates for fungal colonies exhibiting typical color and morphology.

Pour Tube: Melt the pour tube in a boiling water bath and cool to 45-50°C. Mix and dispense into a sterile petri dish and proceed with the instructions above. Aseptically add sheep blood (6% concentration) and/or antimicrobial supplements, if desired.

QUALITY CONTROL
All lot numbers of Brain Heart Infusion Agar w/ and w/o Additives have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing meets or exceeds CLSI Standards.[4] Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
BHI Agar:		
**Candida albicans* ATCC® 10231	Aerobic, up to 72 h @ 25-30°C	Good growth
Cryptococcus neoformans ATCC® 14116	Aerobic, up to 72 h @ 25-30°C	Good growth
Escherichia coli ATCC® 25922	Aerobic, up to 72 h @ 25-30°C	Good growth
Staphylococcus aureus ATCC® 25923	Aerobic, up to 72 h @ 25-30°C	Good growth
**Trichophyton mentagrophytes* ATCC® 9533	Aerobic, up to 72 h @ 25-30°C	Good growth
BHI Agar w/ Chloramphenicol & Cycloheximide:		
**Candida albicans* ATCC® 10231	Aerobic, up to 72 h @ 25-30°C	Good growth
**Trichophyton mentagrophytes* ATCC® 9533	Aerobic, up to 72 h @ 25-30°C	Good growth
**Aspergillus brasiliensis* ATCC® 16404	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
Cryptococcus neoformans ATCC® 14116	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
**Escherichia coli* ATCC® 25922	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)

(Continued on back)

BHI Agar w/ Sheep Blood:

**Candida albicans* ATCC® 10231	Aerobic, up to 72 h @ 25-30°C	Good growth
Cryptococcus neoformans ATCC® 14116	Aerobic, up to 72 h @ 25-30°C	Good growth
**Trichophyton mentagrophytes* ATCC® 9533	Aerobic, up to 72 h @ 25-30°C	Good growth

BHI Agar w/ Sheep Blood w/ Chloramphenicol:

**Candida albicans* ATCC® 10231	Aerobic, up to 72 h @ 25-30°C	Good growth
Cryptococcus neoformans ATCC® 14116	Aerobic, up to 72 h @ 25-30°C	Good growth
**Trichophyton mentagrophytes* ATCC® 9533	Aerobic, up to 72 h @ 25-30°C	Good growth
**Escherichia coli* ATCC® 25922	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)

BHI Agar w/ Sheep Blood w/ Chloramphenicol & Gentamicin:

**Candida albicans* ATCC® 10231	Aerobic, up to 72 h @ 25-30°C	Good growth
Cryptococcus neoformans ATCC® 14116	Aerobic, up to 72 h @ 25-30°C	Good growth
**Trichophyton mentagrophytes* ATCC® 9533	Aerobic, up to 72 h @ 25-30°C	Good growth
**Escherichia coli* ATCC® 25922	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
Pseudomonas aeruginosa ATCC® 27853	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)

BHI Agar w/ Sheep Blood w/ Chloramphenicol, Gentamicin, & Cycloheximide:

**Candida albicans* ATCC® 10231	Aerobic, up to 72 h @ 25-30°C	Good growth
**Trichophyton mentagrophytes* ATCC® 9533	Aerobic, up to 72 h @ 25-30°C	Good growth
**Aspergillus brasiliensis* ATCC® 16404	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
Cryptococcus neoformans ATCC® 14116	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
**Escherichia coli* ATCC® 25922	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
Pseudomonas aeruginosa ATCC® 27853	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)

BHI Agar w/ Sheep Blood w/ Chloramphenicol & Cycloheximide:

**Candida albicans* ATCC® 10231	Aerobic, up to 72 h @ 25-30°C	Good growth
**Trichophyton mentagrophytes* ATCC® 9533	Aerobic, up to 72 h @ 25-30°C	Good growth
**Aspergillus brasiliensis* ATCC® 16404	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
Cryptococcus neoformans ATCC® 14116	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
**Escherichia coli* ATCC® 25922	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)

BHI Agar w/ Sheep Blood w/ Penicillin & Streptomycin:

**Candida albicans* ATCC® 10231	Aerobic, up to 72 h @ 25-30°C	Good growth
Cryptococcus neoformans ATCC® 14116	Aerobic, up to 72 h @ 25-30°C	Good growth
**Trichophyton mentagrophytes* ATCC® 9533	Aerobic, up to 72 h @ 25-30°C	Good growth
**Escherichia coli* ATCC® 25922	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)

BHI Agar w/ Sheep Blood w/ Penicillin, Streptomycin, & Yeast Extract:

**Candida albicans* ATCC® 10231	Aerobic, up to 72 h @ 25-30°C	Good growth
Cryptococcus neoformans ATCC® 14116	Aerobic, up to 72 h @ 25-30°C	Good growth
**Trichophyton mentagrophytes* ATCC® 9533	Aerobic, up to 72 h @ 25-30°C	Good growth
**Escherichia coli* ATCC® 25922	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)

BHI Agar w/ Sheep Blood w/ Penicillin, Streptomycin, & Cycloheximide:

**Candida albicans* ATCC® 10231	Aerobic, up to 72 h @ 25-30°C	Good growth
**Trichophyton mentagrophytes* ATCC® 9533	Aerobic, up to 72 h @ 25-30°C	Good growth
**Aspergillus brasiliensis* ATCC® 16404	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
Cryptococcus neoformans ATCC® 14116	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
**Escherichia coli* ATCC® 25922	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Aerobic, up to 72 h @ 25-30°C	Inhibition (partial to complete)

*CLSI recommended organism

BIBLIOGRAPHY

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2. Rosebury, T., L.J. Epps, and A. R. Clark. 1944. Infect. Dis. 74:131-149.
3. Dolan, C.T. 1971. Appl. Microbiol. 21:195-197.
4. Clinical and Laboratory Standards Institute (CLSI). 2004. Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard, 3rd ed. M22-A3. CLSI, Wayne, PA.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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