Liver Infusion Agar • Liver Infusion Broth

Intended Use

Liver Infusion Agar is used for cultivating *Brucella* and other pathogenic organisms.

Liver Infusion Broth is used for cultivating a variety of organisms, particularly *Brucella* and anaerobes.

Summary and Explanation

Brucellosis is a zoonotic disease with a domestic animal reservoir. Transmission by milk, milk products, meat and direct contact with infected animals is the usual route of exposure.[1]

Most strains of *Brucella* will grow on chocolate or blood agar. However, special media such as liver infusion, tryptose, tryptone or brucella agar are preferred.[2] The nutritive factors of Liver Infusion media permit luxuriant growth of *Brucella* and other fastidious pathogens.

For isolating *Brucella* strains from contaminated milk, crystal violet (gentian violet) can be added to Liver Infusion Agar to suppress gram-positive organisms.[3] Five percent (5%) heated horse or rabbit serum enhances growth of *Brucella*.[4]

User Quality Control

Identity Specifications

Difco™ Liver Infusion Agar

Dehydrated Appearance:	Dark beige to light tan, free-flowing, homogeneous.
Solution:	5.5% solution, soluble in purified water upon boiling. Solution is medium to dark amber, slightly opalescent to opalescent.
Prepared Appearance:	Medium to dark amber, slightly opalescent.
Reaction of 5.5% Solution at 25°C:	pH 6.9 ± 0.2

Difco™ Liver Infusion Broth

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in purified water. Solution is medium to dark amber, clear to very slightly opalescent with a few particles.
Prepared Appearance:	Medium to dark amber, clear to very slightly opalescent with a few particles.
Reaction of 3.5% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ Liver Infusion Agar or Liver Infusion Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours, or up to 72 hours if necessary. Incubate *Clostridium* under anaerobic conditions. Incubate *Brucella* spp. and *S. pneumoniae* with 3-5% CO_2.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Brucella abortus	11192*	30-300	Good
Brucella melitensis	4309*	30-300	Good
Brucella suis	4314*	30-300	Good
Clostridium sporogenes	11437	30-300	Good
Streptococcus pneumoniae	6305	30-300	Good

**Minimally, one strain of* Brucella *should be used for performance testing. These ATCC strains should be used if available.*

Liver Infusion Agar at approximately half strength may be used to prepare Endamoeba medium for cultivating *Endamoeba histolytica*.[5]

Liver Infusion Broth maintains a degree of anaerobiosis well suited to support growth of anaerobic microorganisms, especially *Clostridium* species.

Principles of the Procedure

Peptones and infusions provide the nitrogen, amino acids, vitamins and carbon sources in Liver Infusion media. Sodium chloride maintains the osmotic balance. Agar is the solidifying agent.

Formulae

Difco™ Liver Infusion Agar

Approximate Formula* Per Liter

Beef Liver, Infusion from 500 g	20.0	g
Proteose Peptone	10.0	g
Sodium Chloride	5.0	g
Agar	20.0	g

Difco™ Liver Infusion Broth

Consists of the same ingredients without the agar.

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

Precautions[6]

1. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp.
2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.

Directions for Preparation from Dehydrated Product

1. Suspend/dissolve the powder in 1 L of purified water:
   Difco™ Liver Infusion Agar – 55 g;
   Difco™ Liver Infusion Broth – 35 g.
   Mix thoroughly.
2. Heat the Liver Infusion Agar with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion of the isolation and identification of *Brucella*, anaerobic microorganisms and other fastidious pathogens, refer to the procedures described in *Bailey & Scott's Diagnostic Microbiology*,[4] *Clinical Microbiology Procedures Handbook*[7] and *Manual of Clinical Microbiology*.[8]

Expected Results

Refer to appropriate references and procedures for results.

References

1. Shapiro and Wong. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Carter. 1979. Diagnostic procedures in veterinary bacteriology and mycology, 3rd ed. Charles C. Thomas, Springfield, Ill.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
4. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
5. Cleveland and Sanders. 1930. Arch. Protietenkd. 70:223.
6. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.
7. Isenberg and Garcia. (ed.). 2004. (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
8. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Liver Infusion Agar

Cat. No. 252100 Dehydrated – 500 g

Difco™ Liver Infusion Broth

Cat. No. 226920 Dehydrated – 500 g