Tryptose Agar • Tryptose Broth

Intended Use

Tryptose Agar is used for cultivating a wide variety of fastidious microorganisms, particularly for isolating *Brucella* according to Huddleson and Castañeda.

Tryptose Broth is used for cultivating *Brucella* and other fastidious microorganisms.

Summary and Explanation

Tryptose media, prepared without extract or infusion of meat, are recommended for the cultivation and isolation of pathogenic and saprophytic bacteria. Historically, it was considered necessary to include meat extract or infusion as a nutritional supplement in culture media. Tryptose was developed while studying the growth requirements of *Brucella*. Huddleson[1] found tryptose media to be equal or superior to meat infusion media, providing uniformity for the cultivation and differentiation of fastidious organisms.

Tryptose media are particularly well suited for the isolation of *Brucella* from blood. Castañeda[2] studied the isolation of *Brucella* species using a broth containing 2% tryptose and 2% sodium citrate. Sodium citrate serves as an anticoagulant and assists in inactivating complement in the blood specimen.

Tryptose Broth can be used as a complete basal medium or supplemented with enrichments. Huddleson[3] used a broth containing 2% tryptose as an enrichment medium in the isolation of *Brucella* from clinical specimens. McCullough et al. reported that addition of thiamine, dextrose and iron salts increased growth of *Brucella suis*.[4] Addition of 0.1% agar to Tryptose Broth can increase growth of aerobes and anaerobes in liquid media. Blood agar may be prepared by adding 5% sterile, defibrinated sheep, horse or rabbit blood to the sterile medium.

The high productivity of tryptose media in the isolation and cultivation of *Brucella* supports use of these formulas as general-purpose media, especially when avoidance of animal tissue products is desired. Tryptose Agar with 5% bovine serum, with or without antibiotics, remains a standard plating medium for the isolation of brucellae.[5] For isolation of *Brucella* stains from contaminated milk, crystal violet (gentian violet) can be added to Tryptose Agar to suppress gram-positive organisms.[6] Tryptose media can be supplemented with thiamine or citrate for the cultivation and maintenance of fastidious aerobic and facultative microorganisms.[7]

Tryptose Agar is specified in the *Compendium of Methods for the Microbiological Examination of Foods*.[8] Tryptose media are recommended in the FDA *Bacteriological Analytical Manual* for serological testing.[9]

User Quality Control

Identity Specifications

Difco™ Tryptose Agar

Dehydrated Appearance:	Light beige, homogeneous, free-flowing.
Solution:	4.1% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 4.1% Solution at 25°C:	pH 7.2 ± 0.2

Difco™ Tryptose Broth

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	2.6% solution, soluble in purified water. Solution is light amber, clear.
Prepared Appearance:	Light amber, clear.
Reaction of 2.6% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ Tryptose Agar or Tryptose Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Brucella abortus	11192*	10^2-10^3	Good
Brucella melitensis	4309*	10^2-10^3	Good
Brucella suis	9843*	10^2-10^3	Good

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

Principles of the Procedure

Tryptose peptone is a source of nitrogen and carbon. Dextrose is a source of carbohydrate. Sodium chloride maintains osmotic balance. Agar is the solidifying agent in Tryptose Agar.

Formulae

Difco™ Tryptose Agar

Approximate Formula* Per Liter

Tryptose	20.0	g
Dextrose	1.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

Difco™ Tryptose Broth

Consists of the same ingredients without the agar.

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

Precautions[10]

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

Difco™ Tryptose Agar

1. Suspend 41 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat 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.

NOTE: To prepare blood agar, aseptically add 5% sterile defibrinated sheep, horse or rabbit blood. Dispense into sterile Petri dishes.

Difco™ Tryptose Broth

1. Dissolve 26 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Methodologies for the multiple applications using tryptose media are outlined in the references.

Expected Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Tryptose media are general-purpose, non-selective media. Although certain diagnostic tests may be performed directly on the medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification.
2. When preparing blood agar, hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood.
3. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[11] For optimal performance, incubate tryptose media supplemented with blood under increased CO_2 or anaerobic conditions.
4. Dextrose has been shown to inhibit hemolysin production by some organisms.

References

1. Huddleson. 1943. Brucellosis in man and animals, rev. ed. The Commonwealth Fund, New York, N.Y.
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3. Huddleson. 1939. Brucellosis in man and animals. Oxford University Press, Oxford, England.
4. McCullough, Mills, Herbst, Roessler and Brewer. 1947. J. Bacteriol. *53*:5.
5. Moyer and Holcomb. 1995. *In* Murray, Baron, Pfaller, Tenover, and Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
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8. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods. 4th ed. American Public Health Association, Washington, D.C.
9. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
10. 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.
11. Ruoff, Whiley and Beighton. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Tryptose Agar

BAM CCAM COMPF

Cat. No.	264300	Dehydrated – 500 g
	264100	Dehydrated – 2 kg

Difco™ Tryptose Broth

BAM CCAM COMPF

Cat. No.	262200	Dehydrated – 500 g
	262100	Dehydrated – 10 kg