# **Bacto<sup>™</sup> Tryptose**

## **Intended Use**

**Bacto** Tryptose is an enzymatic digest of protein used in preparing microbiological culture media.

## **Summary and Explanation**

Tryptose was originally developed as a peptone particularly adapted to growth requirements of *Brucella*. Tryptose is very useful for cultivation of streptococci, pneumococci, meningococci and other fastidious organisms, and was found to be superior to meat infusion peptone media previously used for these organisms.<sup>1,2</sup> Mobley et al.<sup>3</sup> reported that Tryptose Broth was the preferred medium for strains of *Bordetella bronchiseptica* in studies of phosphatase activity.

Tryptose has been reported as beneficial for cell culture applications. Litwin<sup>4</sup> found Tryptose to be suitable for supplementing a serum-free medium to grow human diploid fibroblasts. Vaughn and Fan<sup>5</sup> established that Tryptose provided free amino acids necessary for growth of *Spodoptera frugiperda* and *Lymantria dispar* insect cell lines. Tryptose is often used as a biomass enhancer for recombinant *E. coli* production. Tryptose is the major ingredient and only peptone in the formulation for Tryptose Phosphate Broth (TPB), an oftenused medium for various culture applications. Hata and Kojima<sup>6</sup> have shown TPB to be a useful supplement in culturing the nematode, *Angiostrongylus cantonensis*. TPB was also reported as a supplement to a medium for cultivating a protozoan parasite, which parasitizes vectors of Chagas' disease, on its insect cell host.<sup>7</sup> *Spodoptera frugiperda*, a cotton pest in Argentina<sup>8</sup> and several tick cell lines have also been grown using a TPB-supplemented medium.<sup>9</sup> Tryptose Phosphate Broth has been reported as a suitable supplement for growth of baby hamster kidney cells<sup>10</sup> and porcine kidney cells.<sup>11</sup>

Media formulations containing Bacto Tryptose are specified in standard methods for various applications.<sup>12-17</sup>

## **Principles of the Procedure**

**Bacto** Tryptose is a mixed enzymatic hydrolysate with distinctive nutritional properties. The digestive process of **Bacto** Tryptose results in assorted peptides of higher molecular weight suitable for long chain amino acid requirements. **Bacto** Tryptose

## **User Quality Control**

Identity Specifications	
Bacto <sup>™</sup> Tryptose	
Dehydrated Appearance:	Tan, free-flowing, granules.
Solution:	1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is light amber, clear. 2.0% solution is medium amber, clear to slightly opalescent. 10.0% solution is medium to dark amber, very slightly opalescent to opalescent, may have a precipitate.
Reaction of 1.0% Solution at 25°C:	рН 7.1-7.5

#### Cultural Response

## **Biochemical Reactions**

## Bacto<sup>™</sup> Tryptose

Prepare a sterile solution of **Bacto** Tryptose as directed below. Adjust final pH to 7.2-7.4. Inoculate and incubate at  $35 \pm 2^{\circ}$ C for 18-48 hours.

TEST	TEST SOLUTION	ORGANISM	ATCC™	INOCULUM CFU	RESULT
Fermentable Carbohydrates	2%	Escherichia coli	25922	~107	Negative
Indole Production	0.1%	Escherichia coli	29552	0.1 mL, undiluted	Positive
Acetylmethylcarbinol	0.1% with	Enterobacter aerogenes	13048	0.1 mL, undiluted	Positive
Production	0.5% dextrose				
Hydrogen Sulfide Production	1%	Salmonella choleraesuis	14028	0.1 mL, undiluted	Positive
		subsp. choleraesuis serotype Typhimurium			

## Growth Response

### Bacto<sup>™</sup> Tryptose

Prepare a sterile solution with 2% **Bacto** Tryptose, 0.5% sodium chloride and 1.5% agar. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at  $35 \pm 2^{\circ}$ C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Brucella suis	4314*	Undiluted	Good
Staphylococcus aureus	25923	30-300	Good
Streptococcus pneumoniae	6303	30-300	Good
Streptococcus pyogenes	19615	30-300	Good
*If this strain is not available, verify perform	nance with a known isol	ate.	

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provides nitrogen, amino acids and vitamins in microbiological culture media.

## **Typical Analysis**

Refer to Product Tables in the Reference Guide section of this manual.

## **Precautions**<sup>18</sup>

- 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**

Refer to the final concentration of Bacto Tryptose in the formula of the medium being prepared. Add product as required.

## **Procedure**

See appropriate references for specific procedures using Bacto Tryptose.

## **Expected Results**

Refer to appropriate references and procedures for results.

## References

- Casman. 1942. J. Bacteriol. 43:33. Casman. 1947. Am. J. Clin. Pathol. 17:281.
- Mobley, Chengappa, Kadel and Stuart. 1984. Can. J. Comp. Med. 48:175.
  Litwin. 1985. Dev. Biol. Stand. 60:25.
  Vaughn and Fan. 1997. In Vitro Cell. Dev. Biol. Anim. 33:479.

- Hata and Kojima. 1990. Exp. Parasitol. 70:467. Reduth, Schaub, and Pudney. 1989. Parasitology 98:387.
- Deutschmann and Jager. 1994. Enzyme Microb. Technol. 16:506.
- 9. Munderloh and Kurtti. 1989. Exp. Appl. Acarol. 7:219. 10. Prodafikas and Plavsic. 2000. Focus 22:35.
- Alexandre and Fukusho 1998. In Vitro Cell Dev. Biol. Anim. 34:53.
  Horowitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed. AOAC International, Gaithersburg, Md.
- nistration. 1995. Bacteriological analytical manual 8th ed. AOAC 13. U.S. Food and Drug Adm International, Gaithersburg, Md.
- Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C. 15. U.S. Environmental Protection Agency (USEPA). 2000. Improved enumeration methods for the
- recreational water quality indicators: Enterococci and *Escherichia coli*. EPA-821/R-97/004. Office of Water, USEPA, Washington, D.C.
- 16. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C. 17. U.S. Department of Agriculture. 1998. Microbiology laboratory guidebook, 3rd ed. Food and
- Safety Inspection Service, USDA, Washington, D.C.
- U.S. Public Health Service, Conters for Disease Control and Prevention, and National Institutes of Health. 1999. Biosafety in microbiological and biomedical laboratories, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

## Availability

#### Bacto<sup>™</sup> Tryptose

#### AOAC BAM COMPF EPA SMWW USDA

Cat. No. 211713 Dehydrated – 500 g 211709 Dehydrated - 10 kg

## **Tryptose Blood Agar Base**

## Intended Use

Tryptose Blood Agar Base is used with blood in isolating, cultivating and determining the hemolytic reactions of fastidious microorganisms.

## **Summary and Explanation**

Investigations of the nutritive properties of tryptose demonstrated that culture media prepared with this peptone were superior to the meat infusion peptone media previously used for the cultivation of Brucella, streptococci, pneumococci, meningococci and other fastidious bacteria. Casman<sup>1,2</sup> reported that a medium consisting of 2% tryptose, 0.3% beef extract, 0.5% NaCl, 1.5% agar and 0.03% dextrose equaled fresh beef infusion base with respect to growth of organisms. The small amount of carbohydrate was noted to interfere with hemolytic reactions, unless the medium was incubated in an atmosphere of carbon dioxide.

Tryptose Blood Agar Base is a nutritious infusion-free basal medium typically supplemented with 5-10% sheep, rabbit or horse blood for use in isolating, cultivating and determining hemolytic reactions of fastidious pathogenic microorganisms.

Without enrichment, this base can be used as a generalpurpose medium. Tryptose Blood Agar Base is included in the FDA Bacteriological Analytical Manual (pH adjusted to  $6.8 \pm 0.2$ ).<sup>3</sup>

## **Principles of the Procedure**

Tryptose is the source of nitrogen, carbon and amino acids in Tryptose Blood Agar Base. Beef extract provides additional nitrogen. Sodium chloride maintains osmotic balance. Agar is the solidifying agent.

Supplementation with 5-10% blood provides additional growth factors for fastidious microorganisms and is used to determine hemolytic patterns of bacteria.

## Formula

#### Difco<sup>™</sup> Tryptose Blood Agar Base

Approximate Formula* Per Liter	
Tryptose	g
Beef Extract	g
Sodium Chloride5.0	g
Agar	g
*Adjusted and/or supplemented as required to meet performance criteria.	

T Tryptose Blood Agar Base, cont.

#### **User Quality Control** Identity Specifications Difco<sup>™</sup> Tryptose Blood Agar Base Dehydrated Appearance: Beige, free-flowing, homogeneous. Solution: 3.3% solution, soluble in purified water upon boiling. Solution is light amber, very slightly to slightly opalescent. Plain - Light amber, slightly opalescent. Prepared Appearance: With 5% sheep blood - Cherry red, opaque. Reaction of 3.3% Solution at 25°C: pH 7.2 ± 0.2 Cultural Response

#### Difco<sup>™</sup> Tryptose Blood Agar Base

Prepare the medium per label directions without (plain) and with 5% sterile defibrinated sheep blood (SB). Inoculate and incubate at  $35 \pm 2^{\circ}$ C for 18-48 hours (blood plates under 5-10% CO<sub>2</sub>).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB	HEMOLYSIS 18-48 HR
Escherichia coli	25922	10 <sup>2</sup> -10 <sup>3</sup>	Good	Good	Beta
Neisseria meningitidis Stanbulasassus	13090	10 <sup>2</sup> -10 <sup>3</sup>	None to poor	Good	None
aureus	25923	10 <sup>2</sup> -10 <sup>3</sup>	Good	Good	Beta
Streptococcus pneumoniae Streptococcus	6305	10 <sup>2</sup> -10 <sup>3</sup>	Fair to good	Good	Alpha
pyogenes	19615	10 <sup>2</sup> -10 <sup>3</sup>	Fair to good	Good	Beta

## Directions for Preparation from Dehydrated Product

- 1. Suspend 33 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. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium cooled to 45-50°C. Mix well.
- 5. Test samples of the finished product for performance using stable typical control cultures.

## **Procedure**

- 1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions of both oxygen-stable and oxygen-labile streptolysins.<sup>4</sup>
- 2. Incubate plates aerobically, anaerobically or under conditions of increased CO<sub>2</sub> (5-10%) in accordance with established laboratory procedures.
- 3. Examine plates for growth and hemolytic reactions after 18-24 and 48-hour incubation.



## **Expected Results**

Four different types of hemolysis on blood agar media can be described:<sup>5</sup>

- Alpha (α)-hemolysis is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This causes a greenish discolorization of the medium.
- b. Beta  $(\beta)$ -hemolysis is the lysis of red blood cells, resulting in a clear zone surrounding the colony.
- c. Gamma  $(\gamma)$ -hemolysis indicates no hemolysis. No destruction of red blood cells occurs, and there is no change in the medium.
- d. Alpha-prime  $(\dot{\alpha})$ -hemolysis is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

## **Limitations of the Procedure**

- 1. Blood Agar Base Media are intended for use with blood supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.
- Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta-hemolytic on horse, human and rabbit blood agar and alpha-hemolytic on sheep blood agar.<sup>4</sup>

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- 3. Colonies of Haemophilus haemolyticus are beta-hemolytic on horse and rabbit blood agar, and must be distinguished from colonies of beta-hemolytic streptococci using other criteria. The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of H. haemolyticus.<sup>6</sup>
- 4. The atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.<sup>3</sup> For optimal performance, incubate blood agar base media under increased CO<sub>2</sub> or anaerobic conditions.
- 5. Hemolytic patterns may vary with the source of animal blood or type of base medium used.4

## References

- Casman. 1942. J. Bacteriol. 43:33. Casman. 1947. Am. J. Clin. Pathol. 17:281.
- 3.
  - Harmon, Kautter, Golden and Rhodehamel. 1995. In FDA bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md. 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. Isenberg. (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for
- Microbiology, Washington, D.C 6. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, Mo.

## **Availability**

## Difco<sup>™</sup> Tryptose Blood Agar Base

Cat. No. 223220

Dehydrated – 500 g 223210 Dehydrated – 2 kg

# **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.

## **User Quality Control**

<i>Identity Specifications</i> Difco <sup>™</sup> Tryptose Agar	
Dehydrated Appearance:	Light beige, homogeneous, free-flow- ing.
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 <sup>™</sup> 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<sup>™</sup> Tryptose Agar or Tryptose Broth

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

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY		
Brucella abortus	11192*	10 <sup>2</sup> -10 <sup>3</sup>	Good		
Brucella melitensis	4309*	10 <sup>2</sup> -10 <sup>3</sup>	Good		
Brucella suis	9843*	10 <sup>2</sup> -10 <sup>3</sup>	Good		
*Minimally one strain of Brucella should be used for performance testing. These ATCC strains should be used if available.					

## **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<sup>1</sup> 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<sup>2</sup> 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<sup>3</sup> 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.<sup>4</sup> 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.<sup>6</sup> Tryptose media can be supplemented with thiamine or citrate for the cultivation and maintenance of fastidious aerobic and facultative microorganisms.<sup>7</sup>

Section III

Tryptose Agar, cont.

Tryptose Agar is specified in the Compendium of Methods for the Microbiological Examination of Foods.<sup>8</sup> Tryptose media are recommended in the FDA Bacteriological Analytical Manual for serological testing.9

## **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<sup>™</sup> Tryptose Agar

Approximate Formula* Per Liter		
Tryptose		g
Dextrose	1.0	g
Sodium Chloride	5.0	g
Agar		g

#### Difco<sup>™</sup> Tryptose Broth

Consists of the same ingredients without the agar. \*Adjusted and/or supplemented as required to meet performance criteria.

### **Precautions**<sup>10</sup>

- 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<sup>™</sup> 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<sup>™</sup> 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.<sup>11</sup> For optimal performance, incubate tryptose media supplemented with blood under increased CO<sub>2</sub> 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.
- Castañeda. 1947. Proc. Soc. Exp. Biol. Med. 64:114 3
- Huddleson. 1939. Brucellosis in man and animals. Oxford University Press, Oxford, England. McCullough, Mills, Herbst, Roessler and Brewer. 1947. J. Bacteriol. 53:5.
- 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. 5.
- MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. vol. 1. Williams & Wilkins, Baltimore, Md. 7.
- Atlas. 1995. Handbook of microbiology media for the examination of food. CRC Press, Boca Raton. Fla 8. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods
- Downes and no (ed.). 2001. Compensation of interiors for the micropiological examination of foods.
  4th ed. American Public Health Association, Washington, D.C.
  U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC Inter-
- national, Gaithersburg, Md. 10. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of
- Health. 1999. Biosafety in microbiological and biomedical laboratories, 4th 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<sup>™</sup> Tryptose Agar

#### BAM CCAM COMPF

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

#### Difco<sup>™</sup> Tryptose Broth

BAM	CCAM CON	1PF
Cat. No.	262200	Dehydrated – 500 g
	262100	Dehydrated – 10 kg

# **Bacto<sup>™</sup> Tryptose Phosphate Broth**

## **Intended Use**

**Bacto** Tryptose Phosphate Broth is used for cultivating fastidious microorganisms.

## **Summary and Explanation**

Tryptose Phosphate Broth is an infusion-free buffered medium recommended for the cultivation of fastidious, pathogenic microorganisms. It can be used in a procedure for the serodiagnosis of *Listeria monocytogenes*.<sup>1</sup> It is valuable in tissue culture procedures,<sup>2</sup> where the peptone content is considered to be a stimulating factor for cells.

## **Principles of the Procedure**

Peptone provides carbon and nitrogen. Dextrose is a carbon source. Sodium chloride maintains osmotic balance. Buffering capacity is provided by disodium phosphate.

The addition of 0.1-0.2% agar to Tryptose Phosphate Broth facilitates anaerobic growth and aids in dispersion of reducing substances and  $CO_2$  formed in the environment.<sup>3</sup> The low agar concentration provides suitable conditions for both aerobic growth in the upper zone and for microaerophilic and anaerobic growth in the lower zone.

## Formula

## Bacto<sup>™</sup> Tryptose Phosphate Broth

Approximate Formula* Per Liter		
Tryptose	20.0	g
Dextrose	2.0	g
Sodium Chloride	5.0	q
Disodium Phosphate	2.5	g
*Adjusted and/or supplemented as required to meet performance criteria		

## Directions for Preparation from Dehydrated Product

- 1. Dissolve 29.5 g of the powder in 1 L of purified water. (If a medium containing 0.1-0.2% agar is desired, add 1-2 g of agar; heat with frequent agitation and boil for 1 minute to completely dissolve the powder.)
- 2. Autoclave at 121°C for 15 minutes.
- 3. Test samples of the finished product for performance using stable, typical control cultures.

## **Procedure**

See appropriate references for specific procedures.

## **Expected Results**

Refer to appropriate references and procedures for results.

## **User Quality Control**

#### *Identity Specifications* Bacto<sup>™</sup> Tryptose Phosphate Broth

<i>.</i>	
Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.95% solution, soluble in purified water. Solution is light amber, clear to very slightly opalescent, may have a very slight precipitate.
Prepared Appearance:	Light amber, clear to very slightly opal- escent, may have a very slight precipi- tate.
Reaction of 2.95% Solution at 25°C:	pH 7.3 ± 0.2

## Cultural Response

### Bacto<sup>™</sup> Tryptose Phosphate Broth

Prepare the medium per label directions. Inoculate and incubate at 35  $\pm$  2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Neisseria meningitidis	13090	10 <sup>2</sup> -10 <sup>3</sup>	Good
Staphylococcus epidermidis	12228	10 <sup>2</sup> -10 <sup>3</sup>	Good
Streptococcus pneumoniae	6305	10 <sup>2</sup> -10 <sup>3</sup>	Good
Streptococcus pyogenes	19615	10 <sup>2</sup> -10 <sup>3</sup>	Good





Т Tryptose Phosphate Broth, cont.

#### References

- 1. Bennett and Weaver. 1995. In FDA bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
- Ginsberg, Gold and Jordan. 1955. Proc. Soc. Exp. Biol. Med. 89:66. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, 2.
- 3 vol. 1. Williams & Wilkins, Baltimore, Md.

## Tween<sup>™</sup> 80 Water

## **Intended Use**

Tween<sup>™</sup>\* 80 Water may be used to restore and/or inoculate microdilution panels.

\*Tween is a trademark of ICI Americas.

## **Summary and Explanation**

Tween 80 (polysorbate 80) is a surface active agent that is recommended for use at a 0.02% concentration in routine inoculum preparation and dispensing procedures in various microdilution systems.1

## **Principles of the Procedure**

Tween 80 Water is a 0.02% concentration of polysorbate 80 in purified water that is convenient for use in dispersing microorganisms during inoculum preparation and for reconstituting antimicrobial agents in microdilution plates.

## **Tyrosine Agar**

(See Nocardia Differentiation Media)

## **Availability**

Bacto<sup>™</sup> Tryptose Phosphate Broth BAM Cat No 260300 Debydrated – 500 g

.al. NO.	200300	Denyulateu – 500 g
	260100	Dehydrated – 2 kg
	260200	Dehydrated – 10 kg

## **Procedure**

Follow those procedures or test methods requiring the use of water with 0.02% polysorbate 80.

#### Reference

1. Thrupp. 1986. In Lorian (ed.), Antibiotics in laboratory medicine, 2nd ed. Williams & Wilkins, Baltimore, Md.

## **Availability**

## BBL<sup>™</sup> Tween<sup>™</sup> 80 Water

Cat. No.	297381	Prepared Tubes (D Tubes), 12.5 mL – Ctn. of 100
	296207	Prepared Tubes (A Tubes), 25 mL – Pkg. of 10
	206101	Dropared Tubes (A Tubes) 25 ml Ctp of 100

(A Tubes), 25 mL – Pkg. of 10 296184 Prepared Tubes (A Tubes), 25 mL – Ctn. of 100