amino acids and longer-chained peptides. Sodium chloride maintains osmotic equilibrium. Defibrinated sheep blood supplies nutrients necessary to support the growth of fastidious organisms and to detect hemolytic reactions while also inhibiting the growth of *Haemophilus haemolyticus*, a bacterium commonly found in nose and throat specimens that is indistinguishable from beta-hemolytic streptococci.[1] Gentamicin is an aminoglycoside antibiotic that inhibits the growth of gram-negative bacteria. Agar is the solidifying agent.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate the plates in an inverted position (agar side up) at 35°C in a CO_2-enriched atmosphere for 18-48 hours.

Expected Results

Staphylococci and gram-negative bacteria are inhibited. Circular, flat, translucent colonies surrounded by zones of alpha hemolysis may be presumptively identified as *Streptococcus pneumoniae*. However, when the colonies are young, they may be dome-shaped and may be confused with viridans streptococci, which will also grow on this medium. Gram staining, biochemical tests and serological procedures should be performed to confirm findings.

References

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Availability

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) with Gentamicin

Cat. No. 297457 Prepared Plates – Pkg of 20*

**Store at 2-8°C.*

Bacto™ Tryptic Soy Broth/Trypticase™ Soy Broth (Soybean-Casein Digest Medium) Trypticase™ Soy Broth with 6.5% Sodium Chloride Trypticase™ Soy Broth with 5% Fildes Enrichment Bacto™ Tryptic Soy Broth without Dextrose

T

Intended Use

Tryptic (**Trypticase**) Soy Broth (Soybean-Casein Digest Medium) is a general purpose medium used in qualitative procedures for the cultivation of fastidious and nonfastidious microorganisms from a variety of clinical and nonclinical specimens.

Trypticase Soy Broth with 6.5% Sodium Chloride is used to differentiate *Enterococcus* spp. from the *Streptococcus bovis* group of streptococci.

Trypticase Soy Broth with 5% Fildes Enrichment is used for the cultivation of fastidious organisms; e.g., *Haemophilus influenzae*.

Tryptic Soy Broth without Dextrose, a low carbohydrate formulation of Tryptic Soy Broth, is used for cultivating fastidious and nonfastidious microorganisms.

Tryptic (Trypticase) Soy Broth meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

Tryptic (**Trypticase**) Soy Broth (TSB) is a nutritious medium that will support the growth of a wide variety of microorganisms, including common aerobic, facultative and anaerobic bacteria and fungi.[4-7] This formulation is included in the *USP* as a medium for use in performing microbial enumeration tests and tests for specified microorganisms when testing nonsterile pharmaceutical products.[1]

TSB was chosen by the USDA Animal and Plant Health Inspection Service for detecting viable bacteria in live vaccines.[8] TSB is recommended for testing bacterial contaminants in cosmetics[9,10] and complies with established standards in the food industry.[10-16]

Because of its capacity for growth promotion, TSB is also recommended for use as the inoculum broth for disc diffusion and agar dilution antimicrobial susceptibility testing as standardized by the Clinical and Laboratory Standards Institute (CLSI).[17,18]

Trypticase Soy Broth with 6.5% Sodium Chloride is used to differentiate the enterococcal species from the *S. bovis* group of streptococci by the 6.5% NaCl tolerance test.[19]

Trypticase Soy Broth supplemented with 5% Fildes Enrichment provides growth factors necessary for the cultivation of fastidious organisms.[20]

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™/Bacto™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Bacto™ Tryptic Soy Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in purified water upon warming. Solution is light amber, clear.
Prepared Appearance:	Light amber, clear.
Reaction of 3.0% Solution at 25°C:	pH 7.3 ± 0.2

Difco™ Tryptic Soy Broth (prepared bottles)

Appearance:	Light to medium tan yellow, clear to trace hazy.
Reaction at 25°C:	pH 7.3 ± 0.2

Bacto™ Tryptic Soy Broth without Dextrose

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.75% solution, soluble in purified water upon warming. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to very slightly opalescent.
Reaction of 2.75% Solution at 25°C:	pH 7.3 ± 0.2

Uninoculated Tube

Staphylococcus epidermidis ATCC™ 12228

Cultural Response

Bacto™ Tryptic Soy Broth

Prepare the medium per label directions. Inoculate and incubate at 30-35°C for 18-72 hours (up to 5 days for *A. brasiliensis* and *C. albicans*). Prepare duplicate cultures of *A. brasiliensis*, *B. subtilis* and *C. albicans* and incubate at 20-25°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Neisseria meningitidis	13090	10-100	Fair to good
Staphylococcus epidermidis	12228	10-100	Good
Streptococcus pneumoniae	6305	10-100	Good
Streptococcus pyogenes	19615	10-100	Good
Aspergillus brasiliensis (niger)	16404	<100	Growth (30-35°C)
Aspergillus brasiliensis (niger)	16404	<100	Growth (20-25°C)
Bacillus subtillis	6633	<100	Growth (30-35°C)
Bacillus subtillis	6633	<100	Growth (20-25°C)
Candida albicans	10231	<100	Growth (30-35°C)
Candida albicans	10231	<100	Growth (20-25°C)
Escherichia coli	8739	<100	Growth
Pseudomonas aeruginosa	9027	<100	Growth
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	<100	Growth
Staphylococcus aureus	6538	<100	Growth

Difco™ Tryptic Soy Broth (prepared bottles)

Inoculate and incubate at 30-35°C for 18-24 hours (up to 3 days for *B. subtilis*). For (*) cultures incubate at 20-25°C for up to 3 days (up to 5 days for *A. brasiliensis*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
*Aspergillus brasiliensis (niger)**	16404	10-100	Growth (20-25°C)
Bacillus subtillis	6633	10-100	Growth (30-35°C)
*Bacillus subtillis**	6633	10-100	Growth (20-25°C)
*Candida albicans**	10231	10-100	Growth (20-25°C)
Escherichia coli	8739	10-100	Growth
Pseudomonas aeruginosa	9027	10-100	Growth
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10-100	Growth
Staphylococcus aureus	6538	10-100	Growth

Bacto™ Tryptic Soy Broth without Dextrose

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

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Neisseria meningitidis	13090	30-300	Fair to good
Staphylococcus epidermidis	12228	30-300	Good
Streptococcus pneumoniae	6305	30-300	Good
Streptococcus pyogenes	19615	30-300	Good

Continued

Tryptic Soy Broth without Dextrose, a modification of TSB, is a basal medium to which carbohydrates may be added for use in fermentation studies. Phenol red and other indicators may also be added.

Principles of the Procedure

Enzymatic digests of casein and soybean provide amino acids and other complex nitrogenous substances. Dextrose is an energy source. Sodium chloride maintains the osmotic equilibrium. Dibasic potassium phosphate acts as a buffer to control pH.

Identity Specifications

BBL™ Trypticase™ Soy Broth

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 3.0% solution, soluble in purified water upon warming. Solution is light, tan to yellow, clear to slightly hazy.

Prepared Appearance: Light, tan to yellow, clear to slightly hazy.

Reaction of 3.0% Solution at 25°C: pH 7.3 ± 0.2

BBL™ Trypticase™ Soy Broth (prepared bottles)

Appearance: Light to medium tan yellow, clear to trace hazy.

Reaction at 25°C: pH 7.3 ± 0.2

Cultural Response

BBL™ Trypticase™ Soy Broth

Prepare the medium per label directions. Inoculate tubes and incubate at 30-35°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*). Prepare duplicate cultures of *A. brasiliensis, B. subtilis* and *C. albicans* and incubate at 20-25°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	<100	Growth (30-35°C)
Aspergillus brasiliensis (niger)	16404	<100	Growth (20-25°C)
Bacillus subtilis	6633	<100	Growth (30-35°C)
Bacillus subtilis	6633	<100	Growth (20-25°C)
Candida albicans	10231	<100	Growth (30-35°C)
Candida albicans	10231	<100	Growth (20-25°C)
Escherichia coli	8739	<100	Growth
Pseudomonas aeruginosa	9027	<100	Growth
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	<100	Growth
Staphylococcus aureus	6538	<100	Growth

BBL™ Trypticase™ Soy Broth (prepared bottles)

Inoculate and incubate at 35-37°C for 48 hours. Incubate (*) cultures at 30-35°C for up to 3 days. Incubate (**) cultures at 20-25°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	<100	Growth
Staphylococcus aureus	25923	<100	Growth
*Aspergillus brasiliensis (niger)***	16404	<100	Growth (20-25°C)
*Bacillus subtillis**	6633	<100	Growth (30-35°C)
*Bacillus subtillis***	6633	<100	Growth (20-25°C)
*Candida albicans***	10231	<100	Growth (20-25°C)
*Pseudomonas aeruginosa**	9027	<100	Growth (30-35°C)
*Staphylococcus aureus**	6538	<100	Growth (30-35°C)

The addition of 6.5% sodium chloride to **Trypticase** Soy Broth permits the differentiation of salt-tolerant enterococci, which are resistant to the high salt content, from the salt-intolerant *S. bovis* group and other streptococcal species. At this concentration, the sodium chloride is a selective agent that interferes with membrane permeability and osmotic and electrokinetic equilibria.[4]

Fildes Enrichment is a peptic digest of sheep blood that supplies the X (hemin) and V (nicotinamide adenine dinucleotide, NAD) factors necessary for the growth of *H. influenzae*.

Dextrose is omitted from the formula for Tryptic Soy Broth without Dextrose to permit use of the medium in fermentation studies. The carbohydrate concentration used most frequently in fermentation reactions is 0.5% or 1%.

Tryptic Soy Broth and **Trypticase** Soy Broth are provided as prepared media in a variety of bottle styles. In addition, Tryptic Soy Broth is provided as a Sterile Pack Bottle; i.e., the bottle has been terminally sterilized inside of autoclavable double-bags. All varieties of bottled TSB conform with requirements for Ready-To-Use Media as described in the *USP*.

Formulae

Bacto™ Tryptic Soy Broth (Soybean-Casein Digest Medium)

Approximate Formula* Per Liter

Pancreatic Digest of Casein	17.0	g
Papaic Digest of Soybean	3.0	g
Dextrose	2.5	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.5	g

BBL™ Trypticase™ Soy Broth (Soybean-Casein Digest Broth)

Approximate Formula* Per Liter

Pancreatic Digest of Casein	17.0	g
Papaic Digest of Soybean	3.0	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.5	g
Dextrose	2.5	g

Bacto™ Tryptic Soy Broth without Dextrose

Approximate Formula* Per Liter

Pancreatic Digest of Casein	17.0	g
Enzymatic Digest of Soybean Meal	3.0	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.5	g

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

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Bacto™ Tryptic Soy Broth – 30 g;
   BBL™ Trypticase™ Soy Broth – 30 g;
   Bacto™ Tryptic Soy Broth without Dextrose – 27.5 g.
   Mix thoroughly.
2. Warm gently until solution is complete.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.[5,7,17-19]

For food, dairy or cosmetic samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[9-16]

For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.[1]

Procedure

For clinical specimens, refer to appropriate standard references for details on testing protocol to obtain isolated colonies from specimens using Tryptic/**Trypticase** Soy Broth.[17-19]

For food, dairy or cosmetic samples, refer to appropriate standard references for details on test methods using Tryptic/**Trypticase** Soy Broth.[9-16]

For pharmaceutical samples, refer to *USP* General Chapters <61> and <62> for details on the examination of nonsterile products and performing microbial enumeration tests and tests for specific organisms using Tryptic/**Trypticase** Soy Broth.[1]

Swab specimens may be inserted into the medium after inoculation of appropriate plated media. For liquid specimens, use a sterile inoculating loop to transfer a loopful of the specimen to the broth medium. Specimens known or suspected to contain obligate anaerobes should be inoculated near the bottom of the tube.

Incubate the tubes and bottles with loosened caps at 35 ± 2°C aerobically with or without supplementation with carbon dioxide. Tubed and bottled media intended for the cultivation of anaerobes should be incubated under anaerobic conditions. An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak™** EZ anaerobic systems or equivalent alternative system. Examine for growth after 18-24 hours and 42-48 hours of incubation.

Expected Results

Growth in broth media is indicated by the presence of turbidity compared to an uninoculated control. Broth cultures should be held for at least a week before discarding as negative.

References

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Availability

Bacto™ Tryptic Soy Broth (Soybean-Casein Digest Medium)

AOAC BAM BS12 CCAM CLSI CMPH2 COMPF EP EPA ISO JP MCM9 SMD USDA USP

Cat. No.	211824	Dehydrated – 100 g†
	211825	Dehydrated – 500 g†
	211822	Dehydrated – 2 kg†
	211823	Dehydrated – 10 kg†
	290612	Prepared Bottles (wide mouth), 90 mL – Pkg. of 10†
	290613	Prepared Bottles (wide mouth), 100 mL – Pkg. of 10†
	257213	Sterile Pack Bottles (double bagged), 100 mL – Pkg. of 10

Europe

Cat. No.	257423	Prepared Tubes, 13 mL – Pkg. of 25†
	254960	Prepared Bottles (double-strength), 50 mL – Pkg. of 25
	257248	Prepared Bottles, 100 mL – Pkg. of 10†
	257265	Prepared Bottles (double bagged), 100 mL – Pkg. of 10†
	257276	Prepared Bottles, 100 mL (screw cap) – Pkg. of 25†
	257247	Prepared Bottles, 100 mL (tear off seal with stopper) – Pkg. of 25†
	257307	Prepared Bottles (ETO), 100 mL – Pkg. of 44†
	257316	Prepared Bottles (wide mouth), 150 mL – Pkg. of 25†
	257412	Prepared Bottles, 300 mL – Pkg. of 10†
	257413	Prepared Bottles, 500 mL – Pkg. of 4†
	257414	Prepared Bottles, 600 mL – Pkg. of 4†
	257291	Prepared Bottles (double bagged), 800 mL – Pkg. of 4†