TRYPTIC SOY MEDIA
(SOYBEAN-CASEIN DIGEST MEDIA)

PRODUCTS:

Plated Media:[a]

Tryptic Soy Agar	P2500, P3552 (contact plate)
Tryptic Soy Agar with Lecithin and Polysorbate 80	P3500 (contact plate)
Tryptic Soy Agar with Sheep Blood	P2600, P8893 (150mm)
Tryptic Soy Yeast with Rabbit Blood	P2560

Bottled and Tubed Media:[a]

Tryptic Soy Agar[b]	B8800, B8805, T8720
Tryptic Soy Broth	Item no. varies by volume
Tryptic Soy Yeast Broth	T3710, T7750

[a]see catalog for more ordering options

[b]industrial products

PURPOSE:

Tryptic Soy is a general-purpose base media that is used for the cultivation of fastidious microorganisms. It is a Soybean-Casein Digest media and meets the U.S. Pharmacopeia (USP) standards for use in microbiological tests. Slanted tube media, with or without blood, are used for storage and/or shipment of cultures.

PRINCIPLE:

Tryptic Soy is a highly nutritious media and is commonly used as a base media for the cultivation of microorganisms. The addition of blood to Tryptic Soy Agar (TSA) facilitates the growth of more fastidious microorganisms and allows for the interpretation of hemolytic reactions. Hemolytic reactions can be visualized by holding the agar plate in front of a light source or by observing the area surrounding the colony under low power microscopic magnification (10X). Stabbing a loop into the agar several times at the time of media inoculation[4] can enhance hemolysis.

HEMOLYTIC REACTIONS:

Beta Hemolysis	Clear zone surrounding the colony (Complete lysis of the red blood cells).
Alpha Hemolysis	Green discoloration (Partial lysis of the red blood cells).
Alpha Prime Hemolysis	Green discoloration followed by a clear zone.
Gamma Hemolysis	No lysis of the red blood cells surrounding the colony.

Hemolysis may vary with the microorganism and/or with the blood source. Rabbit, horse, and sheep blood are used most frequently. Sheep blood is the generally recommended blood for addition to base agar because beta-hemolytic streptococci show a characteristic clear zone on sheep blood. In addition, *Haemophilus haemolyticus* and *Haemophilus parahaemolyticus* (normal oral flora) appear identical to beta-hemolytic streptococci on horse and rabbit blood and are inhibited on sheep blood.

HEMOLYTIC VARIANCES:	SHEEP	HORSE/RABBIT
Beta-streptococci:		
Groups A,B,C,F,G	Beta/None	Beta/None
Enterococci:**	Alpha/None	Beta/Alpha
Non-enterococci:	Alpha/None	Alpha/None
***Listeria* species:**	Beta/None	Beta/None
***Haemophilus* species:**		
H. influenzae	No growth	None
H. parainfluenzae	No growth	None
H. haemolyticus	No growth	Beta
H. parahaemolyticus	No growth	Beta

** Some strains of *E. faecalis* may show beta hemolysis.[1]

FORMULAS*:

Approximate, per liter of deionized filtered water.

(1) Tryptic Soy Agar (TSA):

Pancreatic Digest of Casein	15.0 g
Enzymatic Soy Digest	5.0
Sodium Chloride	5.0
Agar	15.0

Final pH 7.3 ± 0.2 at 25°C

(2) Tryptic Soy Agar with Sheep Blood:
Same as (1) with various concentrations of Sheep Blood.

(3) Tryptic Soy Yeast Agar with 5% Rabbit Blood and 10.0g of Yeast Extract:
Same as (1) with 50.0 ml of Rabbit Blood and 10.0g of Yeast Extract.
Final pH 7.3 ± 0.2 at 25°C

(4) Tryptic Soy Broth:

Pancreatic Digest of Casein	17.0 g
Enzymatic Soy Digest	3.0
Dextrose	2.5
Sodium Chloride	5.0
Dipotassium Phosphate	2.5

Final pH 7.3 ± 0.2 at 25°C

(5) Tryptic Soy Yeast Broth:
Same as (5) with 10.0 g of Yeast Extract.
Final pH 7.5 ± 0.2 at 25°C

* Adjustments and/or supplements may be required to meet performance standards.

PRECAUTIONS:*

For *in vitro* diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt store plated media at 2-8°C and broth media at 2-25°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, evaporation, or discoloration), or if the expiration date has passed.

Limitations: Tryptic Soy serves as a nonselective media; biochemical and/or serological testing are necessary for the definitive identification of microorganisms isolated.

Tryptic Soy Media may not support the growth of some fastidious microorganisms and the addition of supplements may be required.

Approximately 2% of group A streptococci may be missed if incubated aerobically unless a provision is made to reduce the oxygen tension. It is recommended that several stabs be made into the blood agar at the time of media inoculation. These group A streptococci produce streptolysin O only, an oxygen-labile hemolysin.[3]

Incubation in increased CO_2 is less than ideal for determining streptococcal hemolysis because an increased concentration of CO_2 in the presence of oxygen increases the streptococcal production of peroxide which makes the red blood cells resistant to the lysins of beta-streptococci. Preferably, incubation should be in an anaerobic atmosphere,[1] or aerobically with stabs (see above).

The presence of dextrose in Tryptic Soy Broth (TSB) and Tryptic Soy Yeast Broth (TSYB) make these broths unsuitable for maintaining stock cultures; the fermentation of dextrose by the microorganisms may acidify the media and lead to the destruction of microorganisms. These broths are not typically used for direct inoculation of specimens but are used for the growth enhancement of isolated organisms.

PROCEDURE: *

Specimen Collection: Information on specimen collection is found in standard reference material. In general, specimens should be protected from extreme heat and cold and should be delivered to the laboratory without delay. TSB and TSYB media are used to enhance the growth of isolated organisms.

Method of Use: Prior to inoculation, the media should be brought to room temperature. Inoculate according to standard microbiological procedures and on plate media streak inoculum so as to obtain isolated colonies.

Inoculation and incubation procedures can be found in appropriate referenced guidelines such as USP or CLSI.

Incubate under conditions that will permit the growth of microbes. In general, incubate at 35°C for 1-3 days for bacteria and at 22.5°C for 1-5 days for yeasts and fungi. Incubate with adequate moisture in either aerobic, capnophilic, or anaerobic environments, depending on the specific microbes to be cultured.

Interpretation: The presence of growth in broth media is indicated by turbidity. Colonies on agar media will have diferernt morphologies depending on the isolate.

Organism	Colonial Morphology
Streptococcus pyogenes	Small, beta-hemolytic, transparent to opaque, domed, smooth and entire edge.
Streptococcus viridans	Small, alpha-hemolytic, transparent to opaque, domed, smooth and entire edge.
Streptococcus pneumoniae	Small, alpha-hemolytic, round and mucoid with entire edge.
Staphylococcus aureus	Average, ± hemolysis, opaque, circular, smooth, raised, white to golden yellow pigment.
Staphylococcus epidermidis	Average, ± hemolysis, opaque, circular, smooth, raised, usually white to colorless.
Corynebacteria	Small, grayish colonies.
Listeria monocytogenes	Small, beta-hemolytic, transparent, gray to white.
Yeasts	Small, white to gray in 48-72 hours.

Materials Required but Not Provided: Standard supplies and equipment commonly found in a microbiological laboratory are not provided.

QUALITY CONTROL:*

Media Used:	Microorganisms Used (ATCC #):	Expected Results:
TSA Plain	*Escherichia coli* (8739)	Growth
	Streptococcus pyogenes (19615)	Growth
	Staphylococcus aureus (25923)	Growth
	Haemophilus influenzae (10211)	Growth with XV disk
	Aspergillus niger (16404)†	Growth
	Candida albicans (10231)†	Growth
	Bacillus subtilis (6633)†	Growth
	Pseudomonas aeruginosa (9027)†	Growth
	Staphylococcus aureus (6538)†	Growth
TSA with 5% Sheep Blood	*Streptococcus pneumoniae* (6305)	Growth, alpha hemolysis
	Streptococcus pyogenes (19615)	Growth, beta hemolysis
	Staphylococcus aureus (25923)	Growth
	Escherichia coli (25922)	Growth
TSA with 5% Rabbit Blood	*Streptococcus pneumoniae* (6305)	Growth, alpha hemolysis
	Streptococcus pyogenes (19615)	Growth, beta hemolysis
	Staphylococcus aureus (25923)	Growth
	Escherichia coli (25922)	Growth
	Haemophilus parahaemolyticus (10014)	Growth, beta hemolysis
Tryptic Soy Broth	*Escherichia coli* (25922)	Growth
	Staphylococcus aureus (25923)	Growth
Tryptic Soy Yeast Broth	*Candida albicans* (10231)	Growth
	Streptococcus pyogenes (19615)	Growth

†USP recommended test organism; inoculated with less that 100 colony forming units.

User Quality Control: Check for signs of contamination and deterioration. Tryptic Soy Agar should appear translucent and light tan or straw in color. Tryptic Soy Agar with blood should appear opaque and bright red in color. Tryptic Soy Broth should appear clear and light amber in color.

BIBLIOGRAPHY:

1. Facklam, R. R., *Isolation and Identification of Streptococci*, CDC USHEW, Centers for Disease Control, Atlanta, 1980.
2. Finegold, S. M., and E. J. Baron, *Bailey and Scott's Diagnostic Microbiology*, 7th ed., C. V. Mosby, St. Louis, 1986.
3. Koneman, E. W., et al., *Color Atlas and Textbook of Diagnostic Microbiology,* 3rd ed., J. B. Lippincott, Philadelphia, 1988.
4. Murray, P. R., et al., *Manual of Clinical Microbiology*, 8th ed., American Society for Microbiology, Washington, D. C., 2003.
5. United States Pharmacopeia 30 - NF 25, Chapter 62, Microbial examination of nonsterile products: Tests for specified microorganisms, 2007.

* For more detailed information, consult appropriate references.

PML Microbiologicals, Inc.
Data #780

Revision Date: July 2008