

TRYPTIC SOY AGAR

- For in vitro use only -

<u>Plated Media</u> PT80 –Tryptic Soy Agar (TSA) PT81 – TSA (SXT) PT89 – TSA w Yeast Extract PB75 – TSA w 5% Sheep Blood PB81 – TSA w 7% Sheep Blood PB69 – TSA w 5% Horse Blood PB80 – TSA w 7% Horse Blood

Tryptic Soy Agar (TSA) is a general purpose plating medium used for the isolation, cultivation, and maintenance of a variety of fastidious and nonfastidious microorganisms.

Leavitt et al. demonstrated the versatility of TSA by cultivating both aerobic and anaerobic microbes using TSA. TSA is recognized and recommended by numerous agencies around the world. Our standard formulation is prepared according to the United States Pharmacopeia (USP) and recommended for various different applications put forth by the Association of Official Analytical Chemists (AOAC), the International Dairy Federation (IDF), the United States Department of Agriculture (USDA), and the American Public Health Association (APHA).

Tryptic Soy Agar is a highly nutritious base that meets the growth requirements of many types of microorganisms including bacteria, yeasts, and molds. Many modifications have been to the TSA formulation to increase both its nutritious and selective properties. TSA with 5% defibrinated sheep blood is used extensively for the cultivation and recovery of fastidious microbial species and for the determination of hemolytic reactions that are important differential characteristic especially among the streptococci. This medium is also suitable for performing the overnight CAMP test; Group B streptococci produce an extracellular substance (CAMP factor) that can act synergistically with the beta-toxin produced by some Staphylococcus aureus strains. When placed in close proximity to one another the two organisms produce a zone of increased hemolysis.

<u>Tubed Media</u> TT80 – TSA Slant TT80-18 – TSA Pour Plate [18-mL] TB75 – TSA Blood Slant

The CAMP test can also be used to help identify pathogenic species of *Listeria*.

TSA with horse blood is used to isolate more fastidious organisms. Horse blood contains both X and V factor, which are essential growth factors for some organisms such as *Haemophilus* species. Sheep and human blood are not suitable since they contain specific enzymes that inactivate V Factor.

Although, some laboratories prefer a plated medium with a higher blood content (7-10%) or with horse blood, these mediums should not be used for determination of hemolytic reactions or for the CAMP test. The increased blood content can make hemolytic reactions less distinct and more difficult to read while defibrinated horse blood, in some instances, has shown to give hemolytic reactions different from sheep blood.

TSA supplemented with yeast extract is described in the FDA Bacteriological Analytical Manual for the isolation and purification of *Listeria monocytogenes* as well as other heterotrophic organisms. Gunn, Ohashi, Gaydos, and Holt developed the selective SXT formulation by adding the selective agents sulfamethoxazole and trimethoprim. They found that this medium gave superior isolation of group A and B streptococci from throat specimens by inhibiting the growth of the normal throat flora.

Plain TSA can also be used in the differentiation of *Haemophilus* species when used in conjunction with X, V, and XV factor disks. Differentiation is based on the growth pattern around the various disks.

Formulation per Litre of Medium

PT80 & TT80 Tryptic Soy Agar

Pancreatic Digest of Casein	15.0 g
Papaic Digest of Soybean Meal	5.0 g
Sodium Chloride	5.0 g
Agar	15.0 g

 $pH~7.3\pm0.2$

Additional Ingredients per Liter:

<u>PB81 TSA with 7% Sheep Blood</u> Defibrinated Sheep Blood......70.0 mL

PT81 TSA (SXT)

Defibrinated Sheep Blood	50.0 mL
Sulfamethoxazole	23.75 μg
Trimethoprim	1.25 μg

PT89	TSA	with	Yeast	Extract
110/	1011	vv I tI I	I Cubi	LAnuci

Yeast Extract	g
1 Cubt Lintituet	5

Recommended Procedure

(Please refer to appropriate literature for a more detailed procedure)

- 1. Allow medium to adjust to room temperature prior to inoculation.
- 2. Inoculate by performing a four-quadrant streak on the plated media to obtain well-isolated colonies. For tubed media, streak the surface of the medium in a fishtail motion from bottom up.
- 3. For TSA with blood, several stabs should be made into the medium during inoculation to better detect beta-hemolysis reactions.
- 4. Incubate aerobically or in CO2-rich

environment at 35° C (plates should be inverted).

5. Examine plates and tubes after 18 to 24 hours and at 48 hours.

CAMP Procedure

- 1. Allow medium to adjust to room temperature and ensure that the plate surface is dry prior to inoculation.
- Obtain a pure overnight culture of *Staphylococcus aureus* ATCC 25923 or 33862. With an inoculating needle or edge of a loop streak *Staphylococcus* in a straight line across the center of the plate.
- 3. Streak test organism in a straight line 2 to 3cm long and perpendicular to the staphylococci streak. The line should come close (approx 3mm) but not touch the staphylococci streak. Four test streaks can be performed on each plate although one of the streaks should be a known positive (*Streptococcus agalactiae* ATCC 12386).
- 4. Label the streaks on the bottom of the plate (media side).
- 5. Incubate plates aerobically in an inverted position at 35°C.
- 6. Examine plates after 18 to 24 hours.

Interpretation of Results

TSA with 5% Defibrinated Sheep Blood is commonly used as a primary plating medium. Primary isolation is performed to separate and isolate organisms present in a specimen. This separation allows for characterization of colony types and may indicate the presence of clinically significant bacteria. When examining primary plates a hand lens or stereoscopic microscope should be available for examining very small The different types of colonial colonies. morphology appearing on the agar plate should be noted as well as the number of each morphotype Hemolysis is a useful differential present. characteristic that is best viewed when a bright light is transmitted from behind the plate. Four different types of hemolysis can be described:

- 1. Alpha-hemolysis (α) Partial hemolysis that results in a greenish discoloration around the colony
- 2. Beta-hemolysis (β) Complete lysis of red blood cells resulting in a clear zone around the colony
- 3. Gamma-hemolysis (γ) No hemolysis resulting in no change in the medium
- Alpha-prime-hemolysis (α') A small zone of complete hydrolysis that is surrounded by an area of partial hemolysis

Additional results such as pigment production and odor should also be recorded.

The CAMP test can be used to presumptively identify group B streptococci (*S. agalactiae*). A positive CAMP reaction is defined by the production of a distinct arrowhead zone of complete hemolysis at the point of intersection between the test streak and the *S. aureus* streak. The hemolysis reaction must extend throughout the depth of the agar plate. A negative CAMP reaction is no arrowhead phenomenon or a slight increased zone of hemolysis but with no arrowhead formation. Some organisms such as group A streptococci may show increased hemolysis at the zone of intersection.

Additional tests should be performed on isolated colonies from pure culture in order to complete identification.

- For TSA with sheep blood, stabbing into the medium during inoculation creates an area of reduced oxygen tension that is necessary for hemolysis by oxygen-labile hemolysin O
- Before performing the CAMP test, a betahemolytic organism must be presumptively identified as a member of the genus Streptococcus by a catalase test and gram stain
- Bacteria, other than group B streptococci, may give a positive CAMP reaction, such as Pasteurella haemolytica, Listeria monocytogenes, Burkholderia pseudomallei, Corynebacterium renale, Mobiluncus mulieris, Mobiluncus curtsii, and Propionibacterium

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Results
TSA	
Pseudomonas aeruginosa	Growth
ATCC 27853	
Streptococcus pneumoniae	Growth
ATCC 6305	

TSA w/ 5% Sheep Blood

Streptococcus pneumoniae	Growth, α-hemolysis
ATCC 6305	
Streptococcus pyogenes	Growth, β -hemolysis,
ATCC 19615	CAMP (-)
Streptococcus agalactiae	Growth, β -hemolysis,
ATCC 12386	CAMP (+)
Escherichia coli	Growth
ATCC 25922	

TSA (SXT)

Streptococcus agalactiae	Growth
ATCC 12386	
Streptococcus pyogenes	Growth
ATCC 19615	
Streptococcus pneumoniae	Inhibition
ATCC 6305	
Escherichia coli	Inhibition
ATCC 25922	

TSA w/ Yeast Extract

<i>Listeria monocytogenes</i> Growth ATCC 19114	
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TSA w/ Horse Blood

Haemophilus influenzae	Growth
AICC 10211	
Haemophilus haemolyticus	Growth
ATCC 33390	

Storage and Shelf Life

Our various Tryptic Soy Agar formulations should be stored away from direct light at 4°C to 8°C. For plated media, the medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this mediums have the following shelf lives from the date of manufacture:

PT80 – TSA – 12 weeks

- PT81 TSA (SXT) 8 weeks
- PT89-TSA w Yeast Extract-12 weeks
- PB75 TSA w 5% Sheep Blood 8 weeks
- PB81 TSA w 7% Sheep Blood 8 weeks
- PB69 TSA w 5% Horse Blood 8 weeks
- PB80 TSA w 7% Horse Blood 8 weeks
- TT80 TSA Slant 16 weeks
- TB75 TSA Blood Slant 8 weeks

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