TRYPTIC SOY AGAR

- For in vitro use only -

Plated Media
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

Tubed Media
TT80 – TSA Slant
TT80-18 – TSA Pour Plate [18-mL]
TB75 – TSA Blood Slant

Tryptic Soy Agar (TSA) is a general purpose plating medium used for the isolation, cultivation, and maintenance of a variety of fastidious and non-fastidious 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. 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 ± 0.2

Additional Ingredients per Liter:

PB75 & TB75 TSA with 5% Sheep Blood
Defibrinated Sheep Blood..... 50.0 mL

PB69 TSB with 5% Horse Blood
Defibrinated Horse Blood..... 50.0 mL

PB80 TSA with 7% Horse Blood
Defibrinated Horse Blood..... 70.0 mL

PB81 TSA with 7% Sheep Blood
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
Yeast Extract ..... 6.0 g

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 CO_2–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.
2. 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 colonies. The different types of colonial morphology appearing on the agar plate should be noted as well as the number of each morphotype present. Hemolysis is a useful differential 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
4. 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 beta-hemolytic 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 ATCC 27853	Growth
Streptococcus pneumoniae ATCC 6305	Growth
TSA w/ 5% Sheep Blood	
Streptococcus pneumoniae ATCC 6305	Growth, α-hemolysis
Streptococcus pyogenes ATCC 19615	Growth, β-hemolysis, CAMP (–)
Streptococcus agalactiae ATCC 12386	Growth, β-hemolysis, CAMP (+)
Escherichia coli ATCC 25922	Growth
TSA (SXT)	
Streptococcus agalactiae ATCC 12386	Growth
Streptococcus pyogenes ATCC 19615	Growth
Streptococcus pneumoniae ATCC 6305	Inhibition
Escherichia coli ATCC 25922	Inhibition
TSA w/ Yeast Extract	
Listeria monocytogenes ATCC 19114	Growth
TSA w/ Horse Blood	
Haemophilus influenzae ATCC 10211	Growth
Haemophilus haemolyticus ATCC 33390	Growth

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

References

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