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## TSA Blood Agars

### Tryptic Soy Blood Agar Base No. 2 • Tryptic Soy Blood Agar Base EH • Trypticase™ Soy Agar, Modified (TSA II) Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) Trypticase™ Soy Agar with 10% Sheep Blood (TSA II) Trypticase™ Soy Agar with 5% Horse Blood (TSA II) Trypticase™ Soy Agar with 5% Rabbit Blood (TSA II)

#### Intended Use

Tryptic Soy Blood Agar Base No. 2, Tryptic Soy Blood Agar Base EH and Trypticase Soy Agar, Modified (TSA II) supplemented with blood are used for cultivating fastidious microorganisms and for the visualization of hemolytic reactions produced by many bacterial species.

#### Summary and Explanation

The nutritional composition of TSA (Tryptic Soy Agar/Trypticase Soy Agar) has made it a popular medium, both unsupplemented and as a base for media containing blood. Trypticase Soy Agar, Modified (TSA II) is an improved version of the original TSA formulation for use with animal blood supplements. With 5 or 10% sheep blood, it is extensively used for the recovery and cultivation of fastidious microbial species and for the determination of hemolytic reactions that are important differentiating characteristics for bacteria, especially *Streptococcus* species. Some investigators prefer the use of rabbit or horse blood, but Trypticase Soy Agar with 5% Horse Blood is not recommended for use with throat cultures.

Tryptic Soy Blood Agar Base No. 2 and Tryptic Soy Blood Agar Base EH (enhanced hemolysis) are additional options when hemolytic reactions are important. Tryptic Soy Blood Agar Base No. 2 provides clear hemolytic reactions with group A streptococci, while Tryptic Soy Blood Agar Base EH provides dramatic, improved hemolysis.

Blood agar base media are specified in standard methods for food testing.<sup>1,2</sup>

#### Principles of the Procedure

The combination of casein and soy peptones in Trypticase Soy Agar, Modified (TSA II) renders the medium highly nutritious by supplying organic nitrogen, particularly amino acids and larger-chained peptides. The sodium chloride maintains osmotic equilibrium. Agar is the solidifying agent.

Tryptic Soy Blood Agar Base No. 2 and Tryptic Soy Blood Agar Base EH are similar in composition to TSA II. These formulations utilize the peptones Tryptone H and Tryptone H Plus to enhance hemolysin production while minimizing antagonism or loss in activity of streptococcal hemolysins.

Defibrinated sheep blood is the most widely used blood for enriching agar base media.<sup>3</sup> Hemolytic reactions of streptococci are proper and growth of *Haemophilus hemolyticus*, a nonpathogen whose hemolytic colonies are indistinguishable from those of beta-hemolytic streptococci, is inhibited.

Trypticase Soy Agar with 5% Sheep Blood (TSA II) prepared plates provide excellent growth and beta hemolysis by *Streptococcus pyogenes* (Lancefield group A) and also provide excellent growth and appropriate hemolytic reactions with other fastidious organisms. This medium is suitable for performing the CAMP test for the presumptive identification of group B streptococci (*S. agalactiae*), and for use with low concentration (0.04 unit) bacitracin discs (Taxo™ A) for presumptive identification of group A streptococci (*S. pyogenes*).

The CAMP test is based on the formation of a zone of synergistic hemolysis at the junction of perpendicular streak inocula of *Staphylococcus aureus* and group B streptococci. The reaction is caused by the sphingomyelinase (beta-toxin) of *S. aureus* reacting with sphingomyelin in the sheep erythrocyte membrane to produce ceramide. A non-enzymatic protein (CAMP protein), produced by *S. agalactiae*, binds to the ceramide and leads to disorganization of the lipid bilayer of the sheep erythrocyte membrane resulting in complete lysis.<sup>4</sup>

Trypticase Soy Agar with 10% Sheep Blood (TSA II) prepared plates are provided for those laboratories preferring the increased blood content. This medium is not recommended for performance of the CAMP test. Additionally, the increased blood content can make hemolytic reactions less distinct and more difficult to read.

Trypticase Soy Agar with 5% Horse Blood (TSA II) prepared plates supply both the X and V factors which are growth requirements for certain organisms; e.g., *Haemophilus influenzae*. Sheep and human blood are not suitable for this purpose because they contain enzymes that inactivate the nicotinamide adenine dinucleotide (NAD), which is the V factor.<sup>5</sup>

Defibrinated horse blood may give hemolytic reactions different from sheep blood.<sup>3</sup> Some streptococci (e.g., group D) give hemolytic reactions on horse blood, but not on sheep blood and

## User Quality Control

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

### Identity Specifications

#### Difco™ Tryptic Soy Blood Agar Base No. 2 or Tryptic Soy Blood Agar Base EH

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear to slightly opalescent.
Prepared Appearance:	Plain – Light to medium amber, clear to slightly opalescent. With 5% sheep blood – Bright cherry red, opaque.
Reaction of 4.0% Solution at 25°C:	pH 7.3 ± 0.2

### Cultural Response

#### Difco™ Tryptic Soy Blood Agar Base No. 2\* or Tryptic Soy Blood Agar Base EH

Prepare the medium per label directions with 5% sheep blood. Inoculate and incubate at 35 ± 2°C for 18-48 hours under 5-10% CO<sub>2</sub>.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HEMOLYSIS TSA NO. 2	HEMOLYSIS TSA EH
<i>Escherichia coli</i>	25922	10 <sup>2</sup> -10 <sup>3</sup>	Good	Beta	Beta
<i>Neisseria meningitidis</i>	13090	10 <sup>2</sup> -10 <sup>3</sup>	Good	None	None
<i>Staphylococcus aureus</i>	25923	10 <sup>2</sup> -10 <sup>3</sup>	Good	Beta	Beta
<i>Streptococcus pneumoniae</i>	6305	10 <sup>2</sup> -10 <sup>3</sup>	Good	Alpha	Alpha
<i>Streptococcus pyogenes</i>	19615	10 <sup>2</sup> -10 <sup>3</sup>	Good	Beta	Beta

\*CAMP Test – Perform using *S. aureus* ATCC 25923; *Streptococcus sp. group B* ATCC 12386 (positive) and *S. pyogenes* ATCC 19615 (negative).

### Identity Specifications

#### BBL™ Trypticase™ Soy Agar, Modified (TSA II)

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Plain – Light to medium, yellow to tan, clear to slightly hazy. With 5% sheep blood – Bright red, opaque.
Reaction of 4.0% Solution at 25°C:	pH 7.3 ± 0.2

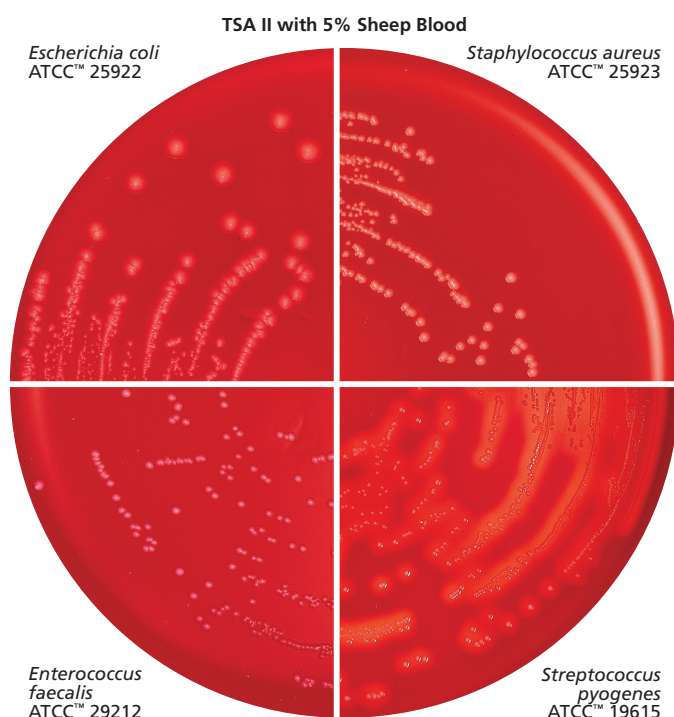
### Cultural Response

#### BBL™ Trypticase™ Soy Agar, Modified (TSA II)

Prepare the medium per label directions with 5% sheep blood. Inoculate and incubate at 35 ± 2°C for 18-48 hours (incubate streptococci with 3-5% CO<sub>2</sub>).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HEMOLYSIS
<i>Candida albicans</i>	10231	10 <sup>3</sup> -10 <sup>4</sup>	Good	None
<i>Escherichia coli</i>	25922	10 <sup>3</sup> -10 <sup>4</sup>	Good, within 24 hours	Beta
<i>Listeria monocytogenes</i>	19115	10 <sup>3</sup> -10 <sup>4</sup>	Good	Beta (+/-)
<i>Shigella dysenteriae</i>	9361	10 <sup>3</sup> -10 <sup>4</sup>	Good	None
<i>Staphylococcus aureus</i>	25923	10 <sup>3</sup> -10 <sup>4</sup>	Good, within 24 hours	Beta
<i>Staphylococcus aureus</i>	6538P	10 <sup>3</sup> -10 <sup>4</sup>	Good	Beta
<i>Streptococcus pneumoniae</i>	6305	10 <sup>3</sup> -10 <sup>4</sup>	Good, within 24 hours	Alpha
<i>Streptococcus pyogenes</i>	19615	10 <sup>3</sup> -10 <sup>4</sup>	Good, within 24 hours	Beta
<i>Streptococcus pyogenes</i>	49117	10 <sup>3</sup> -10 <sup>4</sup>	Good	Beta

CAMP Test – Perform using *S. aureus* ATCC 25923; *Streptococcus sp. group B* ATCC 12386 (positive) and *S. pyogenes* ATCC 19615 (negative).



may be mistakenly reported as group A. If a hemolytic reaction is obtained, the organisms should be tested with a Taxo A disc and grouped serologically or tested by the fluorescent method.<sup>6</sup> Beta-hemolytic streptococci and *Haemophilus hemolyticus* may be differentiated by performing a Gram stain on a smear prepared from the colony.<sup>7</sup>

Defibrinated rabbit blood is also used for enriching agar-based media.<sup>8</sup> Hemolytic reactions on **Trypticase Soy Agar with 5% Rabbit Blood (TSA II)** prepared plates are similar to those on sheep blood. However, rabbit blood does not inhibit *Haemophilus haemolyticus*, a bacterium inhibited by sheep blood that produces colonies indistinguishable from those of beta-hemolytic streptococci.

## Formulae

### Difco™ Tryptic Soy Blood Agar Base No. 2

Approximate Formula* Per Liter	
Tryptone H .....	15.0 g
Soytone .....	5.0 g
Sodium Chloride .....	5.0 g
Agar .....	15.0 g

### Difco™ Tryptic Soy Blood Agar Base EH

Approximate Formula* Per Liter	
Tryptone H Plus .....	15.0 g
Soytone .....	5.0 g
Sodium Chloride .....	5.0 g
Agar .....	15.0 g

### BBL™ Trypticase™ Soy Agar, Modified (TSA II)

Approximate Formula* Per Liter	
Pancreatic Digest of Casein .....	14.5 g
Papaic Digest of Soybean Meal .....	5.0 g
Sodium Chloride .....	5.0 g
Agar .....	14.0 g
Growth Factors .....	1.5 g

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

## Directions for Preparation from Dehydrated Product

1. Suspend 40 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. DO NOT OVERHEAT.
4. For preparation of blood plates, add 5-10% sterile, defibrinated blood to sterile agar which has been cooled to 45-50°C. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

## Procedure

Use standard procedures to obtain isolated colonies from specimens. After streaking, stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to the activity of both oxygen-stable and oxygen-labile streptolysins.<sup>9</sup>

Incubate plates at 35 ± 2°C for 18-72 hours. Since many

pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 5% CO<sub>2</sub>.

### CAMP Test<sup>10</sup>

Non-hemolytic, bile-esculin negative streptococci or bacitracin-resistant beta-hemolytic streptococci may be tested by the CAMP test for presumptive identification as *S. agalactiae* (Lancefield group B). The inoculum may be taken from an overnight broth culture or from colonies picked from a blood agar plate. Make a single streak of *Staphylococcus aureus* ATCC 33862 across the center of a blood agar plate. If a loop is used, do not use it parallel to the agar surface, since the streak will be too wide and the results will not be satisfactory. The streptococcal isolates to be tested are inoculated by making a simple streak perpendicular to the *S. aureus* line coming as close as possible (2-3 mm), but not touching it. Several streptococcal isolates may be tested on the same plate. Perpendicular streptococcal streaks should be 5-8 mm apart. Include a known *S. agalactiae* for a positive control and *S. pyogenes* as a negative control. The procedure should be practiced with known cultures before using it to identify unknown isolates.

NOTE: Studies on the CAMP Test have shown that the reaction is most reliable early in the shelf life of some lots of the prepared plated medium. It is recommended that *S. agalactiae* ATCC 12386 be included along with patient isolates to verify satisfactory performance.

Incubate plates in an aerobic atmosphere at 35 ± 2°C for 18-24 hours. Do not incubate anaerobically or in a CO<sub>2</sub> incubator. False-positive results may occur with group A streptococci when incubation is in an anaerobic or CO<sub>2</sub>-enriched atmosphere.<sup>10,11</sup>

## Expected Results

Hemolytic streptococci may appear translucent or opaque, grayish, small (1 mm), or large matt and mucoid (2-4 mm) colonies, encircled by a zone of hemolysis. Gram stains should be made and examined to check the macroscopic findings. (Other organisms which may cause hemolysis include *Listeria*, various corynebacteria, hemolytic staphylococci, *Escherichia coli* and *Pseudomonas*.) In reporting, approximate quantitation of the number of colonies of hemolytic streptococci may be helpful to the clinician.

- Pneumococci usually appear as very flat, smooth, translucent, grayish and sometimes mucoid colonies surrounded by a narrow zone of "green" (alpha) hemolysis.
- Staphylococci appear as opaque, white to gold-yellow colonies with or without zones of beta hemolysis.
- *Listeria* produce small zones of beta hemolysis. They may be distinguished by their rod shape in stains, and by motility at room temperature.

- *Haemophilus influenzae* produces nonhemolytic, small grayish, translucent colonies with a “mousy” odor on Trypticase Soy Agar (TSA II) with 5% Rabbit Blood.
- Other organisms representing minimal flora and clinically significant isolates can also be expected to grow on this nonselective formulation.

### CAMP Test

A positive CAMP reaction is indicated by an arrowhead or triangular shaped area of increased hemolysis which forms around the end of the streptococcal streak line closest to the *S. aureus* growth. The streptococcal growth must be within the wide zone of partial hemolysis that surrounds the *S. aureus* growth. A negative reaction may appear as a bullet-shaped zone of slightly increased hemolysis or as no increased hemolysis.

Bacitracin-negative, CAMP-positive, beta-hemolytic streptococci may be reported as presumptive group B streptococci. CAMP-positive group A species may be differentiated from group B streptococci by hemolysis, bacitracin susceptibility, and hippurate hydrolysis. Group B streptococci tend to produce larger colonies and have less pronounced zones of beta hemolysis than other beta-hemolytic strains, and some group B strains are nonhemolytic.<sup>9</sup>

### References

1. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
2. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. Vera and Power. 1980. In Lennette, Balows, Hausler and Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
4. Bernheimer, Linder and Avigad. 1979. Infect. Immun. 23:838.
5. Krumweide and Kuttner. 1938. J. Exp. Med. 67:429.
6. Vera. 1971. Health Lab Sci. 8:176.
7. Finegold and Martin. 1982. Bailey & Scott's diagnostic microbiology, 6th ed. The C.V. Mosby Company, St. Louis, Mo.
8. Nash and Krenz. 1991. In Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
9. Ruoff, Whitley and Beighton. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
10. Darling. 1975. J. Clin. Microbiol. 1:171.
11. Facklam and Washington. 1991. In Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

### Availability

#### Difco™ Tryptic Soy Blood Agar Base No. 2

**BAM** **COMPF**

Cat. No. 227300 Dehydrated – 500 g  
227200 Dehydrated – 10 kg

#### Difco™ Tryptic Soy Blood Agar Base EH

**BAM** **COMPF**

Cat. No. 228300 Dehydrated – 500 g  
228200 Dehydrated – 10 kg

#### BBL™ Trypticase™ Soy Agar, Modified (TSA II)

**BAM** **COMPF**

Cat. No. 212305 Dehydrated – 500 g  
297941 Prepared Pour Tubes, 20 mL – Ctn. of 100

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

**BAM** **BS12** **CCAM** **CMPH2** **MCM9** **USDA**

United States and Canada

Cat. No. 221239 Prepared Plates – Pkg. of 20\*  
221261 Prepared Plates – Ctn. of 100\*

Europe

Cat. No. 254053 Prepared Plates – Pkg. of 20\*  
254087 Prepared Plates – Ctn. of 120\*

Japan

Cat. No. 251239 Prepared Plates – Pkg. of 20\*  
251261 Prepared Plates – Ctn. of 100\*  
251240 Prepared Plates – Ctn. of 200\*

#### BBL™ Trypticase™ Soy Agar with 10% Sheep Blood (TSA II)

Cat. No. 221162 Prepared Plates – Pkg. of 20\*  
221260 Prepared Plates – Ctn. of 100\*

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

Cat. No. 221292 Prepared I Plate™ Dishes – Pkg. of 20\*

#### BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//Group A Selective Strep Agar with 5% Sheep Blood (ssa™)

Cat. No. 221783 Prepared Bi-Plate Dishes – Pkg. of 20\*

#### BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//Chocolate II Agar

**BS12** **CMPH2** **MCM9**

United States and Canada

Cat. No. 221302 Prepared I Plate™ Dishes – Pkg. of 20\*  
221303 Prepared I Plate™ Dishes – Ctn. of 100\*

Europe

Cat. No. 251302 Prepared I Plate™ Dishes – Pkg. of 20\*

Japan

Cat. No. 251302 Prepared I Plate™ Dishes – Pkg. of 20\*  
251303 Prepared I Plate™ Dishes – Ctn. of 100\*

#### BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//Levine EMB Agar

**BS12** **CMPH2** **MCM9**

Cat. No. 221286 Prepared I Plate™ Dishes – Pkg. of 20\*  
221289 Prepared I Plate™ Dishes – Ctn. of 100\*

#### BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//MacConkey II Agar

**BS12** **CMPH2** **MCM9**

United States and Canada

Cat. No. 221290 Prepared I Plate™ Dishes – Pkg. of 20\*  
221291 Prepared I Plate™ Dishes – Ctn. of 100\*

Europe

Cat. No. 251290 Prepared I Plate™ Dishes – Pkg. of 20\*

Japan

Cat. No. 251290 Prepared I Plate™ Dishes – Ctn. of 20\*  
251572 Prepared I Plate™ Dishes – Ctn. of 100\*

#### BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//MacConkey II Agar with MUG

Cat. No. 221949 Prepared I Plate™ Dishes – Pkg. of 20\*

#### BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//Chocolate II Agar//MacConkey II Agar

Cat. No. 299580 Prepared Y Plate™ Dishes – Ctn. of 100\*

#### BBL™ Trypticase™ Soy Agar with 5% Horse Blood (TSA II)

United States and Canada

Cat. No. 221372 Prepared Plates – Pkg. of 20\*

Europe

Cat. No. 212099 Prepared Plates – Pkg. of 20\*

#### BBL™ Trypticase™ Soy Agar with 5% Rabbit Blood (TSA II)

Cat. No. 221356 Prepared Plates – Pkg. of 20\*

#### BBL™ Trypticase™ Soy Agar (TSA II) with Defibrinated Sheep Blood Slant

Cat. No. 220830 Prepared Slants – Pkg. of 10\*  
220831 Prepared Slants – Ctn. of 100\*

\*Store at 2-8°C.