



**BBL™ Trypticase™ Soy Agar with 5% Sheep Blood
(TSA II) and Levine EMB Agar - I Plate™**
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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) is used for the growth of fastidious organisms and for the visualization of hemolytic reactions. Levine EMB Agar is a slightly selective and differential medium for the isolation, cultivation and differentiation of gram-negative microorganisms isolated from both clinical and nonclinical specimens. It is widely used for the examination of materials of sanitary importance for the presence of coliforms.

II PERFORMANCE TEST PROCEDURE

A. Trypticase Soy Agar with 5% Sheep Blood (TSA II)

- Inoculate representative samples with dilutions of the cultures listed below.
 - Using a volumetric pipettor or equivalent method, deliver 0.1 mL of a dilution yielding 30–300 CFU to each plate and spread-inoculate using a sterile glass spreader.
 - Incubate the *Staphylococcus* and *E. coli* strains at 35 ± 2°C in an aerobic atmosphere and the *Streptococcus* strains at 35 ± 2°C in an aerobic atmosphere supplemented with 3–5% carbon dioxide.
- Examine plates after 18–24 h for growth, colony size and hemolytic reactions.
- Expected Results

CLSI Organisms	ATCC™	Recovery
* <i>Streptococcus pyogenes</i>	19615	Growth, beta hemolysis
* <i>Streptococcus pneumoniae</i>	6305	Growth, alpha hemolysis
* <i>Staphylococcus aureus</i>	25923	Growth
* <i>Escherichia coli</i>	25922	Growth

*Recommended organism strain for User Quality Control.

B. Levine EMB Agar

- Inoculate representative samples with dilutions of the cultures listed below.
 - Using an 18–24 h broth culture of *Enterococcus faecalis* diluted to yield 10⁴–10⁵ CFU/plate, spread inoculate using a sterile glass spreader. For the remaining organisms, use an 18–24 h broth culture diluted to yield 10³–10⁴ CFU/plate. Streak inoculate *E. coli*. Spread inoculate the remaining organisms.
 - Incubate plates at 35 ± 2°C in an aerobic atmosphere.
 - Include **Trypticase Soy Agar with 5% Sheep Blood (TSA II)** plates as nonselective controls for all organisms.
- Examine plates after 18–24 h for amount of growth, colony size, pigmentation and selectivity.
- Expected Results

CLSI Organisms	ATCC™	Recovery
* <i>Escherichia coli</i>	25922	Growth, blue-black colonies with green metallic sheen
* <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	Growth, colorless to amber colonies
* <i>Enterococcus faecalis</i>	29212	Inhibition (partial)
Additional Organism <i>Shigella flexneri</i>	12022	Moderate to heavy growth. Colonies large, colorless to amber.

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- Examine plates as described under “Product Deterioration.”
- Visually examine representative plates to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for adherence to the specification of 7.4 ± 0.2 (TSA II) and 7.1 ± 0.2 (Levine EMB Agar).
- Note the firmness of plates during the inoculation procedure.
- Incubate uninoculated representative plates aerobically at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Trypticase Soy Agar with 5% Sheep Blood (TSA II) is used for cultivating fastidious microorganisms and for the visualization of hemolytic reactions produced by many bacterial species.

Levine EMB Agar is a selective and differential plating medium for the isolation of gram-negative enteric bacteria.

V SUMMARY AND EXPLANATION

A. Trypticase Soy Agar with 5% Sheep Blood (TSA II)

The nutritional composition of **Trypticase Soy Agar** has made it a popular medium, both unsupplemented and as a base for media containing blood. **Trypticase Soy Agar with 5% Sheep Blood** is extensively used for the recovery and cultivation of

fastidious microbial species and for the determination of hemolytic reactions which are important differentiating characteristics for bacteria, especially *Streptococcus* species.

B. Levine EMB Agar

Shortly following the publication by Holt-Harris and Teague of a paper describing a new culture medium for the differentiation of enteric microorganisms through the use of eosin and methylene blue dyes,¹ Levine described a modification of their formulation which he claimed gave better differentiation between what are now referred to as *Escherichia* and *Enterobacter* species.² The two formulations differ in that Levine EMB Agar does not contain sucrose. Both of these formulations were developed to improve upon the differentiating properties of Endo Agar,³ which was developed previously.

Levine EMB Agar utilizes dyes as selective agents. It is listed for use in the microbiological examination of dairy products and foods by the American Public Health Association.^{4,5}

VI PRINCIPLES OF THE PROCEDURE

A. Trypticase Soy Agar with 5% Sheep Blood (TSA II)

The combination of casein and soy peptones in the **Trypticase Soy Agar** base render the medium highly nutritious by supplying organic nitrogen, particularly amino acids and larger-chained peptides. The sodium chloride maintains osmotic equilibrium.

Defibrinated sheep blood is the most widely used blood for enriching agar base media.⁶ 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) provides excellent growth and beta hemolysis by *Streptococcus pyogenes* (Lancefield group A) and also provides excellent growth and appropriate hemolytic reactions with other fastidious organisms. It is suitable for use with low concentration (0.04 unit) bacitracin discs (**Taxo™ A**) for presumptive identification of group A streptococci (*S. pyogenes*).

B. Levine EMB Agar

The eosin Y and methylene blue dyes in Levine EMB Agar render the medium slightly selective in that they inhibit gram-positive bacteria to a limited degree. These dyes also play a role in differentiating between lactose fermenters and lactose-nonfermenters due to the presence or absence of dye uptake in the bacterial colonies. Coliforms, as lactose fermenting organisms, are visualized as blue-black colonies whereas colonies of *Salmonella* and *Shigella*, as lactose-nonfermenters, appear colorless, transparent or amber in color.

Some gram-positive bacteria, such as fecal streptococci, staphylococci and yeasts, will grow on this medium and usually form pinpoint colonies. A number of nonpathogenic, lactose-nonfermenting gram-negative bacteria will grow on this medium and must be distinguished from the pathogenic strains by additional biochemical tests.

VII REAGENTS

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

Approximate Formula* Per Liter Purified Water

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
Defibrinated Sheep Blood	5%

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

Levine EMB Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin	10.0 g
Lactose	10.0 g
Dipotassium Phosphate	2.0 g
Eosin Y.....	0.4 g
Methylene Blue	0.065 g
Agar.....	15.0 g

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

Warnings and Precautions: For in vitro Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"⁷⁻¹⁰ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2–8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{11,12} Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Trypticase Soy Agar with 5% Sheep Blood (TSA II) and Levine EMB Agar (I Plate)

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate plates, protected from light, at 35 ± 2°C for 18–24 h in an aerobic or CO₂-enriched atmosphere, depending on the source of the specimen or the microorganisms suspected of being present.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After incubation most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen.

Typical results on **Trypticase Soy Agar with 5% Sheep Blood (TSA II)** are as follows:

1. Hemolytic streptococci may appear as translucent or opaque, grayish, small (1 mm), or large matte 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.
2. Pneumococci usually appear as very flat, smooth, translucent, grayish and sometimes mucoid colonies surrounded by a narrow zone of "green" (alpha) hemolysis.
3. Staphylococci appear as opaque, white to gold-yellow colonies with or without zones of beta hemolysis.
4. *Listeria*. Small zones of beta hemolysis are produced. They may be distinguished by their rod shape in stains, and by motility at room temperature.
5. Other organisms representing minimal flora and clinically significant isolates can also be expected to grow on this nonselective formulation.

Typical colonial morphology on Levine EMB Agar is as follows:

- E. coli*Large, blue-black, green metallic sheen
- Enterobacter/Klebsiella*Large, mucoid, blue-black
- Proteus*Large, colorless
- Salmonella*.....Large, colorless
- Shigella*Large, colorless
- Pseudomonas*Irregular, colorless
- Gram-positive bacteriaNo growth to slight growth

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.¹¹⁻¹⁶

XII PERFORMANCE CHARACTERISTICS

Trypticase Soy Agar with 5% Sheep Blood

Trypticase Soy Agar with 5% Sheep Blood was used as a control in a study using broth-enhanced culture (Todd Hewitt) and Optical Immunoassay method for the diagnosis of hemolytic streptococcal infection. Five hundred two (502) specimens were tested. TSA with 5% Sheep Blood had a sensitivity and specificity of 92.5% and 99.4%, respectively.¹⁷ Nguyen et al. used **Trypticase Soy Agar with 5% Sheep Blood** as the "gold standard" for the detection of group B *Streptococcus* from the lower genital tract of pregnant women.¹⁸ In another study, Rossmann et al. successfully reisolated *Lautropia mirabilis* on **Trypticase Soy Agar with 5% Sheep Blood** from the oral cavities of human immunodeficiency virus infected children.¹⁹ Of the 85 children evaluated in this study, 35 (41.4%) were positive for *L. mirabilis*. Isenberg et al. used **Trypticase Soy Agar with 5% Sheep Blood** as a control to evaluate the recovery of *Enterococcus* from a selective medium under study.²⁰ Two hundred fifty (250) group D streptococcal strains isolated from clinical material and 8 strains obtained from the National Communicable Disease Center (Atlanta, Ga.) were used.

XIII AVAILABILITY

Cat. No.	Description
221286	BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) and Levine EMB Agar - I Plate™ , Pkg. of 20 plates
221289	BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) and Levine EMB Agar - I Plate™ , Ctn. of 100 plates

XIV REFERENCES

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