PRODUCT INFORMATION AND QUALITY CONTROL SHEET
TSA 5% SHEEP BLOOD / LEVINE EMB / MASTITIS AGAR TRIPLATE

I. INTENDED USE

Trypticase Soy Agar (TSA) supplemented with sheep blood is an enriched general-purpose medium for the cultivation of microorganisms and the visualization of hemolytic reactions.

Levine EMB Agar is a selective and differential medium for the isolation and differentiation of gram negative enteric bacilli.

Mastitis Agar (or TKT/FC Agar) is a selective and differential medium for the isolation of *Streptococcus* species, especially *Streptococcus agalactiae* associated with bovine mastitis.

II. SUMMARY AND EXPLANATION

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% or 10% sheep blood is used extensively for the cultivation of fastidious microbial species and for the determination of hemolytic reactions which are important differentiation characteristics for bacteria, especially *Streptococcus* species.

Holt-Harris and Teague devised eosine methylene blue agar which, through the use of eosin and methylene blue as indicators, provided good differentiation of lactose fermenting versus non-lactose fermenting microorganisms. Levine modified this original formula by eliminating sucrose and increasing the concentration of agar which provided better differentiation between *Escherichia* and *Enterobacter* species, and reduced the effect of swarming *Proteus* species.

Mastitis Agar was described in 1969 by Fortney in a private communication.[1] It provided a rapid screen to isolate the causative agent of bovine mastitis, *S. agalactiae*, while differentiating it visually from any *Enterococcus faecalis* which may be present in the specimen.[2] This medium was developed based on work by Ward and Postle involving staphylococcal beta-hemolysin and its incorporation into TKT medium.[3]

III. PRINCIPLES OF THE PROCEDURE

The presence of casein and soy peptones in the Trypticase Soy Agar base makes this medium highly nutritious by supplying organic nitrogen, particularly amino acids and larger chained peptides. The sodium chloride maintains osmotic equilibrium. Defibrinated sheep blood, added to enrich the base medium, provides excellent growth and beta hemolysis of *Streptococcus pyogenes* in addition to appropriate hemolytic reactions of other organisms.

The dyes eosin Y and methylene blue act as selective agents by being slightly inhibitory to gram positive bacteria. Further, they enable differentiation between lactose fermenting and non lactose fermenting microorganisms based on the presence or absence of dye uptake in the bacterial colonies. Lactose fermenting coliforms are seen as blue-black colonies and non-lactose fermenting colonies are colorless, transparent, or amber in color. Some gram positive organisms (fecal streptococci, staphylococci and yeasts) will produce pinpoint colonies on this agar.

Mastitis Agar incorporates crystal violet and thallium acetate to inhibit the growth of gram negative organisms and many gram positive organisms. Peptones and yeast extract provide nutrients essential for the growth of streptococci. The presence of sodium chloride maintains osmotic equilibrium. *Streptococcus agalactiae* forms white hemolytic colonies on this medium. *Enterococcus faecalis*, which may grow on this medium, is distinguished by black colonies formed by the splitting of esculin in the presence of ferric citrate

IV. TYPICAL FORMULA AND APPEARANCE

(Approximate formula* per liter of processed water)

TSA 5% SB AGAR

Appearance = opaque, cherry red	
Pancreatic Digest of Casein	14.5g
Papaic Digest of Soybean Meal	5.0
Sodium Chloride	5.0
Agar	14.0
Defibrinated Sheep Blood	5%

LEVINE EMB AGAR

Appearance = wine red with greenish cast, slightly opalescent with fine precipitate	
Pancreatic Digest of Gelatin	10.0g
Lactose	10.0
Dipotassium Phosphate	2.0
Eosine Y	0.4
Methylene Blue	0.065
Agar	15.0

MASTITIS AGAR

Appearance = opaque cherry red	
Pancreatic Digest of Casein	9.0 g
Pancreatic Digest of Animal Tissue	2.7
Yeast extract	2.0
Esculin	1.0
Ferric Citrate	0.05
Sodium Chloride	6.0
Thallium Acetate	0.33
Crystal Violet	0.0013
Agar	13.5
Defibrinated Sheep Blood	5%
Staph hemolysin	10ml

*adjusted and/or supplemented to meet performance criteria.

V. PRECAUTIONS

This product is for IN VITRO diagnostic use only. Culture specimens may contain microorganisms which can be potentially infectious to the user. Strict adherence to aseptic techniques and established precautions against microbiological hazards should be followed throughout the procedure. Carefully dispose of all items which contact patient specimens or isolated bacteria.

VI. STORAGE/SHELF LIFE

Plated media should be stored at 2-8^{0}C (36-46^{0}F), media side up, in the unopened or resealed package protected from light. DO NOT FREEZE OR EXPOSE TO HIGH TEMPERATURES. Allow unopened plates to warm to room temperature prior to inoculation. Prior to and during inoculation procedures, plates should be handled in a manner that minimizes product exposure to the environment. Product which has exceeded the assigned expiration date noted on the label should not be used.

Do not use plates that exhibit evidence of drying, cracking, discoloration, microbial contamination or any other signs of deterioration. The presence of excessive condensate may indicate plates which have been damaged by exposure to temperature extremes.

VII. SPECIMEN COLLECTION

The quality of culture results depends primarily on the adequacy and condition of the specimen submitted for examination. Proper specimen collection techniques must be followed to ensure the most accurate culture results. Sterile swabs and collection containers should be used. Plates should be inoculated promptly after specimen collection. If a delay in inoculation is unavoidable, transport medium should be employed. Specimens should be collected prior to the initiation of antimicrobial therapy.

Detailed information on proper specimen collection may be obtained from microbiology reference materials.

VIII. MATERIALS PROVIDED
TSA 5% SB / Levine EMB / Mastitis Agar Plates

IX. MATERIALS REQUIRED BUT NOT PROVIDED
Incubator maintaining 33-37^{0}C.
Ancillary culture media, reagents and laboratory equipment as required.

X. PROCEDURE
Inoculate the specimen as soon as possible after it is received in the laboratory. The streak plate method is used primarily to isolate pure cultures from specimens containing mixed flora. If material is being cultured directly from a swab, roll the swab over a small area of the plate surface at the edge (approximately 1/4 to 1/3 of the plate); then streak in a zig-zag fashion with a sterile loop from this inoculated area to cover the entire agar surface. Avoid applying excessive pressure to the agar surface during inoculation to prevent gouging and splitting of the agar media. (Note: Agar surfaces should be smooth and moist but free of excessive moisture which could cause confluent growth patterns). Incubate plates media side up at 33-37^{0}C for 24-48 hours in an aerobic atmosphere.

Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere of approximately 3-10% CO_2 (atmospheric incubator or zip-lock bag with generator). Incubate plates media side up at 33-37^{0}C for 18-24 hours.

XI. EXPECTED RESULTS

NCCLS Control Organisms (ATCC Strains)

TSA 5% SB AGAR
Streptococcus pyogenes Growth, beta hemolysis
(ATCC 19615)

Streptococcus pneumoniae Growth, alpha hemolysis
(ATCC 6305)

Staphylococcus aureus Growth
(ATCC 25923)

Escherichia coli Growth
(ATCC 25922)

LEVINE EMB AGAR
Escherichia coli Growth, blue-black colonies
(ATCC 25922) with green metallic sheen

Salmonella typhimurium Growth, colorless colonies
(ATCC 14028)

Enterococcus faecalis Inhibition (partial)
(ATCC 29212)

MASTITIS AGAR
Streptococcus agalactiae Growth, white colonies with
(ATCC 12386) beta hemolysis

Enterococcus faecalis Growth, black colonies
(ATCC 29212)

Escherichia coli No growth to inhibited
(ATCC 25922) growth (partial)

XII. LABORATORY RESULTS
These media are intended to be used as a primary isolation medium. Presumptive identification of organisms may be made on the basis of typical organism morphology and Gram stain. Definitive identification of organisms and antimicrobial sensitivity determination requires further testing. Additional biochemical information may be obtained from reference microbiology texts.[4]

XIII. LIMITATIONS
The ability to detect microorganisms by culture techniques can be affected by the following factors: improper specimen collection, storage and inoculation , initiation of antiinfective therapy prior to specimen collection, improper culture incubation temperatures and atmospheres, improper length of culture incubation, and improper storage and handling of culture media.

XIV. REFERENCES

1. Fortney, R., ARS/Sprague Dawley Co., Private Communication (1969).
2. Ward, Gilbert, and Donald S. Postle, Preparation and Titration of Grade Staphylococcal Beta-Hemolysin for Use in T.K.T. Medium. Journal of Milk and Food Technology, 31:171-173, 1968.
3. Brown, J., R. Farnsworth, L.W. Wannamaker, and D.W. Johnson, CAMP Factor of Group B Streptococci: Production, Assay, and Neutralization by from Immunized Rabbits and Experimentally Infected Cows. Infection and Immunity, 9:377-383, 1974.
4. Lennette, E.H., ed. 1985. Manual of Clinical Microbiology, 4th ed. American Society for Microbiology, Washington, D.C.

TECHNICAL SERVICE

HealthLink provides a toll free technical service line (1-800-638-2625) to assist with product usage. To have technical questions answered; please call between the hours of 9:00 am to 5:00 pm EST.

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