HealthLink
800-638-2625

Product No. 1450
Revision No. 02

PRODUCT INFORMATION AND QUALITY CONTROL SHEET

TSA 5%/EMB/MSA/Mycobiotic Quad Plate

I. INTENDED USE

The TSA 5%/EMB/MSA/Mycobiotic Quad Plate is a four chambered plate containing media designed to assist in the isolation and presumptive identification of a wide variety of microorganisms.

II. SUMMARY AND EXPLANATION

The TSA 5%/EMB/MSA/Mycobiotic Quad Plate contains selective and differential media designed to aid the physician office and alternate laboratory site in the diagnosis of bacterial infections. (i.e., urine, vaginal/cervical, respiratory, etc.).

The TSA 5%/EMB/MSA/Mycobiotic Quad Plate contains an enriched general purpose growth medium (Trypticase Soy Agar with 5% sheep blood), selective and differential agars (Levine's Eosin Methylene Blue Agar, Mannitol Salt Agar, Mycobiotic Agar).

The combination of growth patterns and typical colonial morphologies on these media when compared to standard microbiology reference texts provides a presumptive identification of bacterial or fungal pathogens which may be present in the culture.

III. PRINCIPLES OF THE PROCEDURE

LEVINE'S EMB AGAR

The dyes eosin Y and methylene blue act as selective agents by being slightly inhibitory to gram positive bacteria and 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. (See Product Information Sheet No. 1043 for additional information.)

MANNITOL SALT AGAR

Mannitol Salt Agar is a selective and differential medium for the isolation of staphylococci. It derives its nutritive qualities from the peptone and beef extract content in the formula. These components supply essential growth factors such as nitrogen, carbon, sulfur and trace nutrients. The 7.5% concentration of sodium chloride either completely or partially inhibits the growth of organisms other than staphylococci. The presence of mannitol as a fermentable carbohydrate serves to differentiate *Staphylococci* species. Staphylococci that have the ability to ferment mannitol will produce colonies with yellow zones as indicated by the change in the phenol red indicator. Staphylococci which cannot ferment mannitol will form colonies which do not have yellow zones. (See Product Information Sheet No. 1090 for additional information.)

MYCOBIOTIC AGAR

The nutritive properties of Mycobiotic Agar are supplied by the peptone prepared from soybean meal. Dextrose is an energy source for the metabolism of fungi. Cycloheximide inhibits most saprophytic molds, and Chloramphenicol is a broad-spectrum antibiotic which inhibits a wide range of gram-positive and gram-negative bacteria. (See Product Information Sheet 1109 for additional information).

TRYPTICASE SOY AGAR with 5 % sheep blood

This general growth support medium is highly nutritious. Casein and soy peptones provide organic nitrogen, amino acids and larger peptide chains. The presence of sodium chloride maintains osmotic equilibrium. Defibrinated sheep blood, added to enrich the base medium, provides excellent growth support and enables differentiation of organisms based on hemolytic reactions especially *Streptococci* species. (See Product Information Sheet No. 1160 for additional information.)

IV. TYPICAL FORMULAE AND APPEARANCE

(Approximate formulae* per liter of processed water)

LEVINE'S 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
Eosin Y	0.4
Methylene Blue	0.065
Agar	15.0

MANNITOL SALT AGAR

Appearance = pinkish red, slightly opalescent

Beef Extract	1.0g
Pancreatic Digest of Casein	5.0
Peptic Digest of Animal Tissue	5.0
Sodium Chloride	75.0
D-Mannitol	10.0
Phenol Red	0.025
Agar	15.0

MYCOBIOTIC AGAR

Appearance = light to medium amber, slightly opalescent

Papaic Digest of Soy bean meal	10.0g
Dextrose	10.0
Agar	15.0
Cycloheximide	0.5
Chloramphenicol	0.05

TRYPTICASE SOY AGAR with 5% Sheep Blood

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%

*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°C (36-46°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 and procedural techniques must be followed to ensure the most accurate culture results possible. Specimens should be collected prior to the initiation of antiinfective therapy. Sterile collection containers should be used.

THROAT/PHARYNGEAL SPECIMENS
A properly collected throat swab is essential. Under adequate lighting, depress the tongue and rub a sterile swab vigorously over each tonsillar area and posterior pharynx. Any exudate should be touched, and care should be taken to avoid areas containing large amounts of normal respiratory flora (i.e., tongue, uvula, cheeks, lips, etc.).

URINE SPECIMENS
Urine specimens may be obtained by void, catheterization, or suprapubic aspiration. Voided specimens must be clean catch mid-stream urine. First morning void specimens are preferable. If this is not practical, urine should remain in the bladder for as long as possible before collection. Detailed information on proper specimen collection may be obtained from microbiology reference materials.[1,4]

Plates should be inoculated promptly after specimen collection. For urine specimens, if a delay in inoculation exceeding two hours is unavoidable, specimens may be stored at refrigerated temperatures (2-8°C/36-46°F) in a closed sterile container for a period not to exceed 24 hours.[1,4]

Specimens may contain microorganisms that may be potentially infectious. Strict adherence to aseptic techniques and established precautions should be followed throughout the procedure.

The following recommendations are suggested for additional specimen types or if additional culture information is desired:

SPECIMEN TYPE	PRODUCT NAME	CAT. NO.
Urine	Bullseye Urine Plate	1502
	UniSystem Urine Plate	1552
Respiratory	Respiratory Quad Plate	1405
Cutaneous (for fungus)	Dermatophyte Test Medium	1034

VIII. MATERIALS PROVIDED
TSA 5%/EMB/MSA/Mycobiotic Quad Plates.

IX. MATERIALS REQUIRED BUT NOT PROVIDED
Incubator maintaining 33-37°C.
Sterile specimen collection containers
For Respiratory Specimens: (optional)
Taxo A® differentiating discs
CO_2 kits with bags
Ancillary culture media, reagents and laboratory equipment as required.

X. PROCEDURES

A. URINE CULTURE AND COLONY COUNT
DAY ONE
1. Remove Quad Plate from refrigerated storage and allow to warm to room temperature. Agar surfaces should be free of excessive moisture which could cause confluent organism growth. Label plate with specimen identification number and inoculation date.

2. Resuspend urine specimen by gently swirling container.

3. Immerse a plastic, sterile disposable inoculating loop into the urine specimen up to the loop-shaft junction. Remove loop to obtain a calibrated sample. (Note: Ensure an intact drop of urine is contained within the loop.)

4. Using aseptic technique, transfer the specimen on the loop to the TSA 5% sector. Dispense the drop by touching the loop gently to the agar surface and streak down the center of the entire chamber.

5. Without redipping the loop, zig-zag back and forth over the original streak line multiple times to obtain isolated colonies. (Avoid excess pressure on the inoculation loop which may gouge the media surface.)

6. Repeat Steps 3,4 and 5 for the remaining three chambers.

7. Place the plate, media side up, into the incubator at 33-37° C for 18 - 24 hours.

DAY TWO
1. Remove the plate from the incubator. Remove lid and perform a colony count on all Quad Plates that exhibit bacterial growth as follows: enumerate all colonies on the TSA 5% section, multiply the number of colonies isolated by 100 and record this number as colonies per milliliter.

Example: 120 colonies isolated X 100 = 12,000 col/ml

Note$_1$: For those plates which are negative for bacterial growth, culture report should be interpreted as < 100 col/ml or "no growth".
Note$_2$: Colony counts should be reported for each type of colony morphology present. If multiple colony types are present, the clinician should consider the possibility of a contaminated specimen.

2. Presumptively identify the organism present by comparing the growth reactions of each chamber to reference microbiology texts.

B. THROAT/PHARYNGEAL SPECIMENS

DAY ONE
1. Remove the Quad Plate from refrigerated storage and allow to warm to room temperature. Agar surfaces should be free of excessive moisture which could cause confluent organism growth. Label plate with specimen identification number and inoculation date.

2. Roll the specimen swab, or swab immersed in purulent material, over the initial one third of each agar sector to ensure contact with all swab surfaces. Begin with the TSA 5% chamber followed by the Levine's EMB chamber, MSA chamber and Mycobiotic chamber respectively. Note: If additional test procedures are to be performed (i.e., Gram stain etc.), it is recommended that multiple specimen swabs be collected.

3. Using a plastic sterile disposable inoculating loop, streak in a zig-zag fashion over swabbed areas and continue through the full length of each sector for isolation of colonies. Avoid applying excess pressure to the agar surface during inoculation to prevent gouging and splitting of the agar medium.

4. If testing with differentiation discs (i.e., Taxo A®) is desired on primary inoculum, aseptically place one disc on the TSA 5% chamber. (See also Section XIII. LIMITATIONS)

5. Incubate the plate at 33-37°C for 18 - 48 hours. Note: Incubation under CO_2 atmosphere if desired may be accomplished by ordering HL Cat. No. 3425, CO_2 kit. If using a CO_2 kit, insert the inoculated plate and a CO_2 generator into a zip-lock bag. Seal the bag, crush the generator in an upright position and allow the liquid contained in the vial to contact the pellets. When effervescing has stopped, place the unopened zip-lock bag into the incubator media side up.

6. Pathogens may be detected as early as 18 hours, however, it is recommended that inoculated plates be incubated for a total of 48 hours before reporting a culture as "normal flora".

DAY TWO
1. Remove the plate from the incubator. (Note: The CO_2 atmosphere within the zip lock bag will dissipate upon opening the bag. Examination of culture results prior to detection of organism growth or 48 hours of incubation may be accomplished by; 1) examining the plate through the unopened bag or 2) using an additional CO_2 generator upon reincubating the opened and examined culture plate (Catalog #3425).

2. Presumptively identify the suspected pathogenic organism(s) present by comparing the growth reactions of each chamber to reference microbiology texts.

3. Interpretation of Taxo A® Zones of Inhibition

3.1 Taxo A®: Observe the TSA 5% chamber for growth of beta hemolytic streptococci. Any zone of inhibition of beta hemolytic streptococci around the disc should be interpreted as positive.[4] Interpretation is as follows:
Zone present = "beta hemolytic *Streptococci*, presumptive group A by bacitracin"
Zone absent = "beta hemolytic *Streptococci* species, presumptively not group A by bacitracin.

XI. EXPECTED RESULTS

NCCLS CONTROL ORGANISMS (ATCC STRAINS)

Levine's EMB Agar

Escherichia coli (25922) - Growth, blue-black colonies with green metallic sheen
Pseudomonas aeruginosa (27853) - Growth, colorless colonies, irregular edges
Enterococcus faecalis (29212) - Inhibition (partial)

Mannitol Salt Agar

Staphylococcus aureus (25923) - Growth, colonies have yellow zones at 48 hours
Staphylococcus epidermidis (12228) - Growth, colonies are white, may have slight pink zones
Proteus mirabilis (12453) - Inhibition (partial)

Mycobiotic Agar

Candida albicans (10231) - Growth
Tricophyton mentagrophytes (9533) - Growth
Escherichia coli (25922) - Inhibition (partial to complete)

TSA with 5% sheep blood

Streptococcus pyogenes (19615) - Growth, beta hemolysis
Streptococcus pneumoniae (6305) - Growth, alpha hemolysis
Escherichia coli (25922) - Growth
Staphylococcus aureus (25923) - Growth

HealthLink conforms to all NCCLS recommendations for quality control of commercially prepared microbiological media where applicable.

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.

Definitive identification of certain organisms requires additional testing which may include; Gram stain, oxidase, catalase, and other biochemical tests. Additional information on organism identification can be found in microbiology reference materials.[2,3,4]

Interpretation of Taxo A® tests for presumptive identification of *S. pyogenes* are based on tests involving pure culture. Taxo A® discs placed on nonselective media inoculated directly with throat swabs presumptively identify between 50 - 97% of group A Streptococci.[5] Furthermore, many alpha hemolytic Streptococci, including *S. pneumoniae*, are susceptible to bacitracin.

Isolation of pathogenic or potentially pathogenic organisms does not necessarily indicate involvement in the disease process. These organisms may also be detected in the "carrier" state. Various respiratory pathogens (i.e. *S. pneumoniae, H. influenzae,* members of the *Enterobacteriaceae*, and *S. aureus*) may be transient or minor components of the oropharyngeal flora in healthy individuals without consequent development of infection. Because of this, the physician must often make diagnostic decisions on etiological suspicion in addition to culture results.[5]

Some fungi may be inhibited by the antibiotics contained in Mycobiotic Agar. Cultures for fungi other than *Candida albicans* need to be inoculated onto a non selective fungal medium such as Sabouraud Dextrose (HL Cat. No. 1137). Dual incubation temperatures may be required. Cultures for dermatophytes and agents of superficial mycoses should be inoculated onto Dermatophyte Test Medium (DTM - HL Cat. No. 1034) and incubated according to the product information sheet. Appropriate reference materials should be consulted for appropriate processing and inoculation of specific specimens.[6]

To obtain antimicrobial susceptibility results on presumptively identified bacterial urinary tract pathogens, order HL Cat. No. 1103/1106, Mueller Hinton Agar.

XIV. REFERENCES

1. Clarridge, J.E., M.T. Pezzlo, K.L. Vosti. March 1987. Cumitech 2A. Laboratory Diagnosis of Urinary Tract Infections. Coordinating ed., A.S. Weissfeld. American Society for Microgbiology, Washington, D.C.
2. Finegold, S.M. and W.S. Martin. 1982. Bailey and Scott's Diagnostic Microbiology, 6th ed. C.V. Mosby Company, St, Louis.
3. Koneman, E.S., S.D.Allen, V.R. Dowell, Jr. and H. M. Sommers. 1983. Color Atlas and Textbook of Microbiology, 2nd ed. J.B. Lippincot Company, Philadelphia.
4. Lennette, E.H., ed. 1985. Manual of Clinical Microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
5. Bannatyne, R.M., C. Clausen, L.R. McCarthy. 1979. Cumitech 10. Laboratory Diagnosis of Upper Respiratory Tract Infections. Coordinating ed., I.B.R. Duncan. American Society for Microbiology, Washington, D.C.
6. Ajello, L., L.K. Georg, W. Kaplan, and L. Kaufman. 1963. CDC Laboratory Manual for Medical Mycology. PHS Publication No. 994, U.S. Government Printing Office, Washington, D.C.

USER QUALITY ASSURANCE/ QUALITY CONTROL PROCEDURES AND INFORMATION

HealthLink recommends that the following quality assurance and quality control procedures be performed on each batch of product.

I. QUALITY ASSURANCE

The following quality assurance procedures must be performed to assure the product will perform according to its intended use within the assigned expiry date:

1. Daily, document that product storage refrigerator maintains temperature within the recommended range: 2-8°C.
2. Daily, document that laboratory incubator maintains temperature within the recommended range: 22-35°C.

II. QUALITY CONTROL

The following incoming inspection procedures must be performed for each batch (batch = same lot, same shipment) of culture media received in the laboratory :

1. Inspect plates according to instructions contained on the "Quality Control Log Sheet."
2. Peel off the lower portion of a product bag label (Quality Control Certificate) for the lot being accepted into the laboratory and affix it to the Quality Control Log Sheet.
3. Initial and date the Quality Control Log Sheet.

Note: Notify Technical Service immediately if media does not meet the inspection criteria.

TECHNICAL SERVICE

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

HealthLink
3611 St. Johns Bluff Rd. So. Ste. 1
Jacksonville, FL 32224
1-800-638-2625
September, 1999

Product No. 1450 Rev. No. 02