

PRODUCT INFORMATION AND QUALITY CONTROL SHEET

EMB/XLD/Citrate Urinary/TSA 5% Agar Quad Plate

I. INTENDED USE

The EMB/XLD/Citrate Urinary / TSA 5% Agar Quad plate is a four chambered plate containing media designed to assist in the isolation and presumptive identification of bacterial urinary tract pathogens.

II. SUMMARY AND EXPLANATION

The EMB/XLD/Citrate Urinary / TSA 5% Agar Quad Plate was designed to contain the same media combination found in the Bullseye Urine Culture System. The Bullseye Culture System originated in the mid 1970s to aid the physician office and alternate laboratory site in the diagnosis of bacterial infections.

Each type of culture plate in the Bullseye system was designed to contain media directed towards the isolation and presumptive identification of bacterial pathogens based on clinical specimen site (i.e., urine, vaginal/cervical, respiratory, etc.).

The EMB/XLD/Citrate Urinary/TSA 5% Agar Quad Plate contains an enriched general purpose growth medium (Trypticase Soy Agar with 5% sheep blood), selective and differential agars (Levine EMB, Citrate Urinary Agar, Modified XLD agar).

The combination of growth patterns and typical colonial morphologies on these media when compared to standard microbiology reference texts provides a colony count and presumptive identification of the bacterial pathogen present.*

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.)

MODIFIED XLD AGAR

Modified XLD Agar is a selective and differential agar for gram negative enteric organisms. Traditional XLD agar is recommended for the isolation of *Salmonella* and *Shigella* sp. from clinical materials containing large amounts of normal flora.

In the Modified XLD formulation, the selective agent sodium desoxycholate has been reduced to permit the growth of enteric organisms normally inhibited by the traditional formula concentration. Organisms that ferment xylose, lactose or sucrose will form colonies with yellow zones in the presence of the phenol red indicator. Organisms that decarboxylate lysine, an alkaline reaction, will form colonies with red zones. Organisms that produce hydrogen sulfide produce black centered colonies in the presence of the indicator system consisting of sodium thiosulfate and ferric ammonium citrate.

CITRATE URINARY AGAR

Organisms able to utilize ammonium dihydrogen phosphate and sodium citrate as the sole sources of nitrogen and carbon respectively will grow on this medium and produce an alkaline reaction. These colonies in the presence of the bromthymol blue indicator will produce a color change in the medium from green to blue.

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. (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

MODIFIED XLD AGAR

Appearance = red, slightly opalescent, no precipitate

Xylose	3.75g
L-lysine	5.0
Saccharose	7.5
Lactose	7.5
Sodium chloride	5.0
Yeast extract	4.0
Sodium desoxycholate	0.5
Sodium thiosulfate	7.0
Ferric ammonium citrate	1.5
Phenol red	0.08
Agar	15.0

CITRATE URINARY AGAR

Appearance = forest green, slightly opalescent, may have slight precipitate

Ammonium dihydrogen phosphate	1.0g
Potassium phosphate dibasic	0.5
Sodium phosphate tribasic	0.5
Sodium chloride	4.0
Sodium citrate	3.0
Magnesium sulfate	0.2
Bromthymol blue	0.08
Agar	15.0

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

* The Bullseye Urine Identification Chart may also be used as an identification aid when using the TSA 5%/EMB/XLD/Citrate Urinary Agar Quad Plate.

Plated media should be stored at 2-8^oC (36-46^oF), 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 anti-infective therapy. Sterile collection containers should be used.

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. If a delay in inoculation exceeding two hours is unavoidable, specimens may be stored at refrigerated temperatures (2-8^oC/36-46^oF) 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.

VIII. MATERIALS PROVIDED

EMB/XLD/Citrate Urinary/TSA 5% Agar Quad Plates
Disposable inoculating loops
Patient Report Pad

IX. MATERIALS REQUIRED BUT NOT PROVIDED

Incubator maintaining 33-37^oC.
Sterile specimen collection containers
Ancillary culture media, reagents and laboratory equipment as required.

X. PROCEDURE

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^oC 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%, 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: For those plates which are negative for bacterial growth, culture report should be interpreted as < 100 col/ml or "no growth".

Note: 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 or the Bullseye Urine Identification Chart.

Note: The Bullseye Urine Identification Chart is a full color wall chart containing photographic representation of organism growth. Each agar contained in the Quad Plate is identical to those contained in the Bullseye Urine. All media sectors are identical with the exception of the chamber or sector shape. Whereas the Bullseye Urine Plate sectors are "arc shaped", the Quad Plate sectors are "pie shaped." (See TECHNICAL SERVICE Section for ordering information.)

XI. EXPECTED RESULTS

URINE INSTRUCTIONAL KIT (Cat. #3122)

SECTOR #	E. coli (ATCC 25922)	P. aeruginosa (ATCC 27853)
1 - Levine's EMB	Growth, blue/black colonies with green metallic sheen.	Growth, transparent to colorless irregular colonies.
2 - Modified XLD	Growth, yellow colonies with red to yellow media.	Growth, colorless colonies with magenta media.
3 - Citrate Urinary	Inhibition (partial) green to blueish/green media.	Growth, clear to opalescent colonies with blue media.
4 - TSA 5%	Growth, large gray colonies.	Growth, large irregular colonies.

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 anti-infective 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 test. Additional information on organism identification can be found in microbiology reference materials.^{2,3,4}

Over incubation of culture plates (>24 hours) may cause culture overgrowth leading to difficulty in interpretation of colony count and possible inaccuracy of biochemical reactions.

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 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.

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2. Finegold, S.M. and W.S. Martin. 1982. Bailey and Scott's Diagnostic Microbiology, 6th ed. C.V. Mosby

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." (See also Section VI "STORAGE/SHELF LIFE")
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 additional QA/QC log sheets, or to have technical questions answered; please call between the hours of 9:00 am to 5:00 pm EST.

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