PRODUCT INFORMATION AND QUALITY CONTROL SHEET
BULLSEYE URINE PLATE

I. INTENDED USE
The Bullseye Urine Plate is a five-chambered plate containing media designed to assist in the isolation and presumptive identification of bacterial urinary tract pathogens and the determination of their antimicrobial susceptibility patterns.

II. SUMMARY AND EXPLANATION
The Bullseye culture system was designed in the mid 1970s to aid the physician office and alternate laboratory site in the diagnosis of bacterial infections.

The Bullseye Urine 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), and Mueller Hinton agar which is the recommended medium for antimicrobial susceptibility testing by the disc method.

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 in addition to a presumptive antimicrobial susceptibility pattern.

III. PRINCIPLES OF THE PROCEDURE
SECTOR 1 - 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.)

SECTOR 2 - 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.

SECTOR 3 - 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.

SECTOR 4 - 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.)

CENTER SECTOR - Mueller Hinton Agar
In the 1960s, a wide range of media and procedures were used in clinical microbiology laboratories to determine susceptibility patterns of bacteria to various antimicrobial agents. Bauer, Kirby and others selected Mueller Hinton Agar as the medium of choice to develop a standardized procedure for the determination of susceptibility patterns by the disc diffusion method. An international study confirmed the value of Mueller Hinton medium for this purpose due to its reproducibility, simplicity of formula and large experimental database.

IV. TYPICAL FORMULAE AND APPEARANCE
(Approximate formulae* per liter of processed water)

SECTOR 1 - 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
Eosine Y	0.4
Methylene Blue	0.065
Agar	15.0

SECTOR 2 - 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

SECTOR 3 - 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
Brom thymol blue	0.08
Agar	15.0

SECTOR 4 - Trypticase Soy Agar with 5% Sheep Blood
Appearance = opaque, cherry red

Pancreatic Digest of Casein	15.0g
Papaic Digest of Soybean Meal	5.0
Sodium Chloride	5.0
Agar	15.0
Defibrinated Sheep Blood	5%

CENTER SECTOR - Mueller Hinton Agar
Appearance = pale amber, slightly opalescent

Beef Extract	2.0g
Acid Hydrolysate of Casein	17.5
Starch	1.5
Agar	17.0

*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 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.

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^{0}C/36-46^{0}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.

VIII. MATERIALS PROVIDED

Bullseye Urine Plates (10 ea.)
Disposable inoculating loops (10 ea.)
Patient Report Pad (10 ea.)

IX. MATERIALS REQUIRED BUT NOT PROVIDED

Bullseye Urine Identification Chart
Incubator maintaining 33-37^{0}C.
0.5 McFarland Standard
Tubed sterile saline
Antibiotic sensitivity disc cartridges
Disc dispenser (6 or 8 place)
Measuring device in millimeters
Sterile specimen collection containers
Ancillary culture media, reagents and laboratory equipment as required.

X. PROCEDURE

DAY ONE

1. Remove Bullseye Urine 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 Sector #4. 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 4 and 5 for the remaining three outside chambers.

7. Place the plate, media side up, into the incubator at 33-37^0 C for 18 - 24 hours.

DAY TWO

1. Remove the plate from the incubator. Remove lid and perform a colony count on all Bullseye Urine plates that exhibit bacterial growth as follows: 1) Estimated colony count: compare the growth on Agar Sector 4 or Sector 1 to the five representative colony count photographs at the top of the Bullseye Urine Identification Chart. Select the photograph that most closely matches the culture growth observed. 2) Calculated colony count: 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 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 morpohology 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*.

3. Indirect Susceptibility Test

Prepare a "Standardized" inoculum by picking up 3 to 5 well isolated colonies of the same morphological type from Sector 1 or 4 with a sterile inoculation loop or swab and transfer to a tube containing approximately 5 ml of sterile saline to achieve the turbidity of the 0.5 McFarland Standard. Tubes should be visually compared under adequate light.

4. If needed adjust the turbidity of the culture with sterile saline or additional colonies to obtain turbidity visually comparable to that of the 0.5 McFarland Standard.

5. Within 15 minutes after turbidity adjustment, dip a sterile swab into the suspension and rotate it firmly against the tube wall above the fluid level to express excess fluid.

6. Inoculate the surface of the Mueller Hinton section by streaking the swab over the entire agar surface. Repeat this procedure two more times, rotating the plate approximately 60° each time to ensure even inoculum distribution. Note: Mueller Hinton plates should be free of excessive moisture prior to inoculation.

7. Place evenly spaced antibiotic susceptibility discs onto the center section using sterile forceps or a disc dispenser. Gently tap discs to ensure complete contact with the agar surface. (Note: Do not move discs once contact with the agar surface has been made.)

8. Invert the plates and place them in a 35-37^{0}C incubator for 16 - 18 hours within 15 minutes after the discs are applied. Note: Plates should not be incubated in an atmosphere of increased carbon dioxide.

DAY THREE

1. Antimicrobial Susceptibility Pattern*

3.1 Measure the zone of inhibition surrounding each antibiotic susceptibility disc. This can be achieved with either the Bullseye Urine Zone Overlay or a measuring device calibrated in millimeters.

3.2 Compare the measured zones to either the instructions on the Bullseye Urine Zone Overlay or Table 2 of the NCCLS Standard

M2-A5, "Performance Standards for Antimicrobial Disk Susceptibility Tests."

*See also LIMITATIONS Section for additional information.

XI. EXPECTED RESULTS

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.

Mueller Hinton (See USER QUALITY CONTROL Section)

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 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 and antibiotic sensitivity results.

Direct Susceptibility testing is not a standardized disc method procedure as outlined by the National Committee for Clinical Laboratory Standards (NCCLS). However as described in a 1973 study by Perez and Gillenwater, there is a high degree of correlation between direct susceptibility testing using infected urine as the inoculum when compared to the standardized disc susceptibility method.[5,6] The results of this study were evaluated by Hollick and Washington in 1976 and the following recommendations were outlined and must be considered when reporting a direct susceptibility test result: 1) reports should not be relied upon if more than one colony type is present, 2) results should be reported only on those specimens with colony counts in excess of 100,000 col/ml, 3) If the patient does not respond to initial treatment, an indirect susceptibility test should be performed as a follow up procedure.[7]

XIV. REFERENCES

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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. Perez, J. R. and J.Y. Gillenwater, 1973. Clinical Evaluation of Testing Immediate Antibiotic Disk Sensitivities. Journ. of Urol. Vol.110:452-456.

6. Waterworth, P.M., and M. Del Piano. 1976. Dependability of sensitivity tests in primary culture. Journ. of Clin. Path. Vol 29:179-184.

7. Hollick, Gary E., and J. A. Washington. 1976. Comparison of Direct and Standardized Disk Diffusion Susceptibility Testing of Urine Cultures. Antimicrobial Agents and Chemotherapy. Vol. 9, No. 5: 804-809.

8. National Committee for Clinical Laboratory Standards. 1993. Approved Standard: M2-A5. Performance Standards for Antimicrobial Disk Susceptibility Tests, 5th ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.

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^{0}C.
2. Daily, document that laboratory incubator maintains temperature within the recommended range: 33-37^{0}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 free technical service line (800-638-2625) to assist with product usage. To have technical questions answered, call between the hours of 9:00 am to 5:00 pm EST.

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