

Procedure: Uricult® CLED/EMB

Prepared by	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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INTENDED USE:

Uricult is a simple, self-contained urine test method for the detection of bacteria that commonly cause urinary tract infections.

PRINCIPLE:

In the mid 1960's, Mackey and Sandys developed a dip inoculum transport medium for rapid urine culturing. Subsequent improvements have been made which increase the accuracy of the results obtained while retaining the convenience of the dip inoculum technique.

URICULT Urine CULTURE-PADDLES® provide effective bacterial detection and presumptive identification in a simple and reliable manner.

URICULT Urine CULTURE-PADDLES are attached to a screw cap. Each side of the culture-paddle is coated with an agar medium suitable for the growth of urinary bacteria, and the culture-paddle is suspended in a clear plastic vial. The CULTURE-PADDLES are safely isolated in this vial during transport, incubation, storage and handling. Because of the uniform application of agar to the URICULT Urine CULTURE-PADDLE, it is possible to obtain semi-quantitative results when the device is used as directed. This is determined by a simple visual comparison of bacterial growth on the agar surface with the Colony Density Chart provided. No actual colony counting is necessary.

EQUIPMENT AND MATERIALS:

Materials:

Provided in the kit:

10 Paddles
Package Insert
Patient ID Stickers

Required but not provided in the kit:

Small incubator calibrated to maintain a temperature of $36^{\circ}\text{C} \pm 2^{\circ}\text{C}$
Urine collection cup

Preparation:

None

Performance Parameters:

URICULT Urine CULTURE-PADDLES are for IN VITRO DIAGNOSTICA USE.

Do not use product beyond expiration date.

Do not use URICULT Urine CULTURE-PADDLES exhibiting discoloration, dehydration of the agar, media separating from the paddle or evidence of mold or bacterial growth.

Because bacterial colonies on inoculated URICULT Urine CULTURE-PADDLES are actual or potential pathogens, a potential biohazard may exist and the CULTURE-PADDLES should not be touched or unduly exposed.

Storage Requirements:

Store Uricult at $45^{\circ}\text{F} - 77^{\circ}\text{F}$ ($7^{\circ}\text{C} - 25^{\circ}\text{C}$) in the package provided. Protection from light and temperature fluctuations will ensure product stability to the expiration date. Avoid drafts and do not store near heat-generating appliances.

DO NOT FREEZE.

QUALITY CONTROL:

- A. This product was tested and found to meet the NCCLS Approved Standard for commercially prepared media as required by CLIA '88 regulations.

Quality control tests are performed on each lot of URICULT Urine CULTURE-PADDLES at the time of manufacture. Media quality is assessed through a "use-test" performed using

bacterial strains obtained from clinical urine specimens. This test verifies the ability of the media to support the growth of bacteria most commonly isolated from cases of urinary tract infection. The ability of the media to exhibit the expected biochemical reactions and colony morphology is also verified.

Should the user wish to perform their own Quality Control, the following procedure is recommended.

The following organisms are tested by the manufacturer and meet the Quality Control Criteria according to the NCCLS Approved Standard for:

- a. *Escherichia coli* ATCC 25922
- b. *Proteus Mirabilis* ATCC 12453
- c. *Proteus vulgaris* ATCC 8427
- d. *Salmonella typhimurium* ATCC 14028
- e. *Enterococcus faecalis* ATCC 29212
- f. *Staphylococcus aureus* ATCC 25923

The laboratory shall remove and file the “Certificate of Analysis” statement provided by the manufacturer as part of package insert for each lot of Uricult and file:

_____.

- B. Upon receipt of each shipment of Uricult, the laboratory shall perform a visual inspection of two vials from each box of Uricult to assure it has not been damaged. **The vials shall be inspected for the following characteristics and reported on the “Quality Control Report” attached hereto:**

Cracked Vials	Unequal filling of paddles
Cracked Media	Excessive number of bubbles in Media
Freezing (adverse temperatures)	Contamination
Discoloration of Media	Media Separated from Paddle (Media Off)
Dehydration of Media	Excess Moisture Greater than 2millimeters on bottom of vial

The Quality Control Report shall be filed:_____

_____.

SPECIMEN:

Ideally, urine for culture analysis should be incubated in the bladder for four hours prior to collection. Urine samples may be obtained by voiding, catheterization or supra-pubic aspiration. If a voided specimen is to be used, a mid-stream, clean catch specimen is recommended.

Specimens should be inoculated onto URICULT Urine CULTURE-PADDLES immediately following collection. CULTURE-PADDLES should be immediately replaced in the protective vial, loosely replacing the screw cap.

If storage of urine specimens is necessary, the specimens should be maintained at refrigerator temperature (4°C/39°F) in a closed sterile container. Storage should not exceed two hours.

PROCEDURE - STEPWISE:

Compliance with the following directions is required to achieve reliable test results.

1. Remove the URICULT Urine CULTURE-PADDLES from the protective vial by unscrewing the vial cap.
2. *Handling the URICULT Urine CULTURE-PADDLES by the cap*, dip the CULTURE-PADDLE into the urine specimen to fully immerse the agar surfaces. If the urine volume is not adequate to fully immerse the agar surfaces, as is sometimes the case with infants or small children, the urine may be poured over the agar surfaces.
3. Allow the excess urine to drain from the URICULT CULTURE-PADDLE. The base of the culture-paddle may be blotted on absorbent paper if desired.
4. Replace the inoculated URICULT Urine CULTURE-PADDLE in its protective vial.
5. Complete patient label indicating patient's name, date, and time of inoculation. Attach label to URICULT vial.
6. Place inoculated URICULT vial upright in incubator (36°C±2°C) for 18 to 24 hours. Incubation should not exceed 24 hours. Incubation exceeding 24 hours may cause bacterial overgrowth resulting in difficult interpretation of colony counts and possibly misleading biochemical reactions.
7. Remove URICULT vial from incubator following incubation period. Compare colony count density on the agar surfaces with the Colony Density Chart provided to obtain a semiquantitative colony count in CFU/ml of urine. Compare only the number of colonies present, not the size of the colonies or the agar surface area they cover. The colonies on the agar surface may also be observed at this time for morphology and agar color reactions which may be used for presumptive identification of the bacterial growth.

REPORTING RESULTS:

Reference Ranges:

When the recommended procedure for a clean catch, mid-stream specimen collection is followed, contamination of the specimen is minimized. Kass has recommended the following guidelines for interpretation of urinary colony counts on voided specimens:

NORMAL - Less than 10,000 CFU/ml urine

DOUBTFUL - 10,000 to 100,000 CFU/ml urine

POSITIVE - Greater than 100,000 CFU/ml urine

Reporting Format:

Following the incubation of an inoculated URICULT Urine CULTURE-PADDLE, the presence of bacteria may be evidenced by visible signs of colony growth on the agar surface. Separate, distinct areas of bacterial growth on the agar surface are called "colonies". Since the formation of a colony results from the natural multiplication of a single bacterial cell, and since the agar surfaces on URICULT Urine CULTURE-PADDLES are uniform in dimension, the number of colonies can indicate the "colony count" which is the approximate number of CFU/ml of urine.

At the end of the incubation period, check the agar surfaces on both sides of the URICULT Urine CULTURE-PADDLE for colony growth. If all visible bacterial colonies are similar in characteristics, compare the number of colonies on each side of the culture-paddle. If there is a significant difference in the number of colonies on each side, the side with the greater number should be used for determining the "colony count". In making the determination, the number of colonies and not the dimensions of the individual colonies should be considered. Match the "colony density" on the agar surface with the printed example it most closely resembles on the Colony Density Chart. If the characteristics of visible colonies on either side of the culture-paddle differ enough to indicate more than one type of bacteria, the colony count match-up procedure should be performed and reported for each organism.

"Confluent growth" (complete coverage of the agar surfaces) may occasionally occur when a colony count is more than 100,000 (10^5) CFU/ml, and *may be misinterpreted as a negative culture* because there is no clear definition between colonies. To avoid misinterpretation, it is recommended, therefore, that cultures which appear to have no clearly defined colonies be scanned under a bright light. The light will be reflected from the agar surface when there are no bacterial colonies. An agar surface completely coated with confluent bacterial growth will not reflect the light. The use of bright light will also allow relatively small colonies to be seen.

Further confirmation of a negative culture may be obtained by gently swabbing part of the agar surface. Bacterial growth will be evident on the swab itself, and by a difference in appearance between the swabbed and unswabbed portions of the agar surface.

The determination of colony color, size, texture, configuration, and observation of the agar media for color change induced by bacterial growth, can provide information useful in making a presumptive identification of the bacteria present.

Reference sources should be consulted for expected colony morphology and biochemical reactions of the various bacterial species frequently encountered in urine specimens.

LIMITATIONS OF THE PROCEDURE:

URICULT Urine CULTURE-PADDLES are capable of detecting bacteriuria concentrations as low as 1,000 CFU/ml of urine. The Colony Density Chart allows the reporting of colony counts to the nearest power of 10. When used as directed, comparison of URICULT colony count results with conventional pour plate methods shows an overall correlation of 99%.

Bacterial identifications based on the biochemical reactions evidenced by URICULT and colony morphology will result only in a presumptive identification. Bacterial variation may occur and atypical strains may be isolated. In instances where a definitive bacterial identification is necessary for proper patient management, the inoculated paddle may be used as a transport media to forward the bacterial specimen to a laboratory for further study.

Panic Values

There are no imminent life-threatening laboratory results or panic values for urine cultures.

PROCEDURES FOR REPORTING PATIENT RESULTS:

REFERENCES:

1. Mackey JP, Sandys GH: Laboratory Diagnosis of Infections of the Urinary Tract in General Practice by Means of a Dip-Inoculum Transport Medium. *British Medical Journal* **2**:1286-1288, 1965.
2. Kass EH: Bacteriuria and the Diagnosis of Infections of the Urinary Tract. *Arch.Int.Med.* **100**:709-714, 1957.
3. McAllister TA, Arneil GC, Barr W, Kay P: Assessment of Plain Dipslide Quantitation of Bacteriuria. *Nephron* **11**:111-122, 1973.