Certificate of Analysis

Uricult CLED+Polymyxin/EMB • Product No. 1002

year-month-day

pH range	Growth results		CLED	EMB	Lactose reaction
CLED+Pol. 6.7–7.3	*Escherichia coli*	ATCC 25922	Inhibited	Growth	Pos.
	Proteus vulgaris	ATCC 8427	Growth	Variable	Neg.
	Salmonella typhimurium	ATCC 14028	Inhibited	Growth	Neg.
EMB 6.7–7.3	*Enterococcus faecalis*	ATCC 29212	Growth	Small colonies	Pos.
	Staphylococcus aureus	ATCC 25923	Growth	No growth	Pos.
	Pseudomonas aeruginosa	ATCC 27853	Inhibited	Growth	Neg.

This lot meets the Quality Control Criteria set by Aidian Oy for microbial load (contamination).
This EMB lot meets the CLSI Approved Standard for commercially prepared media,
see package insert Quality control section for CLED+Polymyxin media.

Signature:

Intended use

10 Urine Culture-Paddles® for the detection of bacteriuria and the presumptive identification of uropathogens. The test is intended for *in vitro* diagnostic use.

Summary and explanation of the test

In the mid 1960's, Mackey and Sandys developed a dip inoculum transport medium for rapid urine culturing[1]. 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.

Principles of the procedure

CLED-Polymyxin Agar (Cystine-Lactose-Electrolyte-Deficient/Polymyxin)
CLED medium was first described by Mackey and Sandys[1] specifically for use in dip inoculum procedures for urinary bacteriology. The electrolyte deficient nature of the medium prevents the characteristics swarming of *Proteus*. The inclusion of lactose and the indicator dye, Brom-Thymol Blue, allows differentiation of lactose fermenting bacteria, since the by-products of lactose fermentation cause the color of the agar to change from its original pale green color toward yellow.
The incorporation of Polymyxin into the CLED medium creates a selective medium for the growth of *Enterococci*, *Staphylococci*, *Proteus*, and *Yeast*, preventing the growth of most *E. coli*, *Klebsiella*, and *Enterobacter*. Additionally, *Pseudomonas* colony growth is inhibited. Occasionally, mutant colonies of *E. coli*, *Klebsiella*, and *Enterobacter* will be observed on this medium as large mucoid colonies.

EMB Agar (Eosin Methylene Blue)
EMB agar is a selective reddish-brown colored agar recommended for the detection and isolation of Gram negative intestinal pathogenic bacteria. The balanced combination of the two dyes (Eosin and Methylene Blue) contained in the EMB agar makes it possible to distinguish lactose fermenting bacteria from non-fermenters. Those bacteria which ferment lactose will have dark colonies, purple or black in color. A green sheen may be observed across the agar surface during the growth of certain lactose fermenting bacteria.

Reagents

Contents

Uricult® CLED+Polymyxin/EMB	Product No. 1002
Urine CULTURE-PADDLES® (in individual vials)	10
Patient labels	10
Instructions for use containing the Colony Density Chart	1

An incubator calibrated to maintain a temperature of 97°F ± 4°F (36°C ± 2°C) is necessary but not provided.

Typical formulation

CLED-Polymyxin medium			EMB medium		
Peptone	10.0	g/L	Peptone	10.0	g/L
Meat extract	3.0	g/L	Lactose	5.0	g/L
Lactose	10.0	g/L	Sucrose	5.0	g/L
L-Cystine	0.13	g/L	Dipotassium Phosphate	2.0	g/L
Brom-Thymol Blue			Eosin Y		
Polymyxin			Methylene Blue		

Storage
- Store at 45...77°F (7...25°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.

Warnings and precautions

- URICULT® Urine CULTURE-PADDLES® are for ***in vitro* diagnostic** use only.
- 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.

Specimen collection and preparation

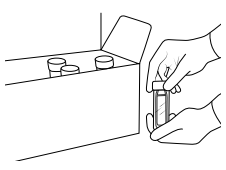
Ideally, urine for culture analysis should be incubated in the bladder for four hours prior to collection. Urine samples may be obtained by voiding, catheterization or supra-pubic aspiration. If a voided specimen is to be used, a mid-stream, clean catch specimen is recommended.
Specimens should be inoculated onto URICULT® Urine CULTURE-PADDLES® immediately following collection. The CULTURE-PADDLES® should be immediately replaced in the protective vial, closing the screw cap. The inoculated CULTURE-PADDLE® may be incubated immediately or stored or transported to a laboratory for incubation and/or interpretation.
If stored or transported to a laboratory, the URICULT® cap should be tightened. Storage or transportation should not exceed 48 hours at 45...77°F (7...25°C). Stored or transported Uricult should be incubated at 97°F ± 4°F (36°C ± 2°C) for 18–24 hours. URICULT® paddles which have been stored or transported up to 48 hours before incubation can only be used for growth and/or colony count.
Transportation of URICULT® Urine CULTURE-PADDLES® with tight caps may result in inconclusive agar color reactions and atypical colony morphology, making presumptive identification impossible.
The laboratory should inspect the inoculated URICULT® CULTURE-PADDLE® upon arrival to assure the CULTURE-PADDLE® was not frozen during transit. In instances where freezing is suspected, a new specimen should be obtained and transported to the laboratory for evaluation.
If storage of urine specimens prior to inoculation is necessary, the specimen should be maintained at refrigerator temperature (36…46°F/2…8°C) in a closed sterile container. Storage should not exceed 24 hours.

Note
URICULT® test results may be affected if the patient has been receiving antibiotics or anti-infective treatment. Should URICULT® test results show the presence of bacterial growth during the course of therapy, the physician may wish to reassess the dosage or organism susceptibility to the anti-infective being used.
URICULT® testing may be used to assess the effectiveness of antibiotic or anti-infective therapy. In this instance, it is recommended that a test be performed no sooner than 48 hours (2 days) following the administration of the final dose of medication.

Procedure

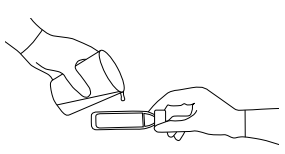
Compliance with the following directions is required to achieve reliable test results.

1



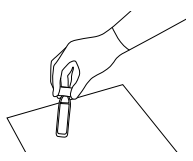
Remove the URICULT® Urine CULTURE-PADDLES® from the protective vial by unscrewing the vial cap.

2



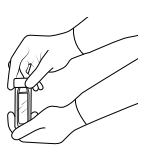
Handling the URICULT® Urine CULTURE-PADDLE® *by the cap*, dip the CULTURE-PADDLE® into the urine specimen to fully immerse the agar surfaces. If the urine volume is not adequate to fully immerse the agar surfaces, as is sometimes the case with infants or small children, the urine may be poured over the agar surfaces.

3



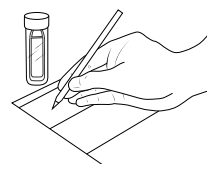
Allow the excess urine to drain from the URICULT® Urine CULTURE-PADDLE®. The base of the culture-paddle may be blotted on absorbent paper if desired.

4



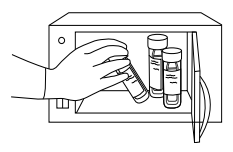
Replace the inoculated URICULT® Urine CULTURE-PADDLE® in its protective vial.

5



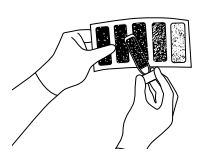
Complete patient label indicating patient's name, date and time of inoculation. Attach label to URICULT® vial.

6



Place inoculated URICULT® vial upright in incubator 97°F ± 4°F (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.

8

Negative cultures may be incubated for an additional 24 hour period, if desired. This will allow for the detection of slow growing bacteria.

Quality control

This product was tested and found to meet the CLSI 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.

The user is required to Quality Control each lot or shipment of Uricult containing CLED Polymyxin media according to the CLSI approved Standard and CLIA ´88. This testing should demonstrate the selectivity of the CLED Polymyxin media to support growth, inhibition, lactose fermentation and elimination of *Proteus* swarming.

The following organisms may be used to demonstrate the performance characteristics of the CLED + Polymyxin media:

Characteristic	Recommended	Alternative
No growth (inhibition)	*E. coli*	*Pseudomonas*
Growth & lactose positive	*S. aureus*	*E. faecalis*
Growth, lactose negative & no swarming	*P. vulgaris*	*P. mirabilis*

Suitable ATCC strains for QC can be found on the Certificate of Analysis.

The following test procedure is recommended:
1. Prepare separate suspensions of the following organisms in sterile normal saline and adjust to a concentration of 10^4 to 10^5 CFU/mL, so that isolated colonies are provided:
   a. *Escherichia coli* *ATCC 25922*
   b. *Proteus vulgaris* *ATCC 8427*
   c. *Salmonella typhimurium* *ATCC 14028*
   d. *Enterococcus faecalis* *ATCC 29212*
   e. *Staphylococcus aureus* *ATCC 25923*

NOTE: We recommend that the test organisms be prepared in the manner specified by the American Type Culture Collection.

2. Inoculate each bacterial suspension onto a separate URICULT® Urine CULTURE-PADDLE® and incubate in the manner described in the test procedure section of this insert.
3. Interpret the results following 24 hour incubation as follows:
   ***Escherichia coli* ATCC 25922**: No growth should be seen on the CLED+ Polymyxin medium. A maximum of five colonies is an acceptable result. Growth of purple or metallic green colonies on the EMB medium.
   ***Proteus vulgaris* ATCC 8427**: Growth of translucent colonies with a color change toward blue on the CLED+Polymyxin medium.
   ***Salmonella typhimurium* ATCC 14028**: No growth should be seen on the CLED+Polymyxin medium. A maximum of five colonies is an acceptable result. Growth of colorless colonies on the EMB medium.
   ***Enterococcus faecalis* ATCC 29212**: Growth of yellow colonies with a color change toward yellow on the CLED+Polymyxin medium and growth of pin point colonies on the EMB medium.
   ***Staphylococcus aureus* ATCC 25923**: Growth of yellow colonies on the CLED+Polymyxin medium only.

Results' interpretation

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 the 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 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 changes 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%[3].
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 incubated paddle may be used as a transport media to forward the bacterial culture to a laboratory for further study.
Incubation of the inoculated URICULT® CULTURE-PADDLE® at 97°F ± 4°F (36°C ± 2°C) should not exceed 48 hours.
In instances where the inoculated paddle cannot be incubated and read within 48 hours, the URICULT® paddle may be stored or transported at 45...77°F (7...25°C) for 48 hours after tightening the URICULT® caps and placing the vial in an area protected from direct sunlight and temperature fluctuations. Following storage or transport, inoculated URICULT® paddles should be incubated at 97°F ± 4°F (36°C ± 2°C) for 18–24 hours. URICULT® paddles which have been treated in this manner can only be used for colony count and growth. Inoculated paddle storage up to 48 hours with caps tightly closed may result in inconclusive agar color reactions and atypical colony morphology, making a presumptive identification not possible. In instances where a definitive bacterial identification is necessary for proper patient management, the incubated paddle may be used as a transport media to forward the bacterial culture to a laboratory for further study.

Expected values

When the recommended procedure for a clean catch, mid-stream specimen collection is followed, contamination of the specimen is minimized. Kass[2] has recommended the following guidelines for the 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

Many factors such as use of antimicrobial therapy, time of bladder incubation of the specimen, and means of specimen collection may influence the colony count obtained. In all cases, the physician must be the final judge of the proper interpretation of the URICULT® results.

Performance characteristics

URICULT® Urine CULTURE-PADDLES® are capable of detecting bacteriuria in the range of 10^3 (1000) to 10^7 (10,000,000) CFU/mL.
In a published study[3] involving 340 clean catch, mid-stream urine specimens, the agreement between URICULT® and the pour plate method was 99%.
No false negatives resulted from the use of URICULT®. Three specimens provided positive results with URICULT® but were negative by the pour plate technique; therefore, URICULT® demonstrated a specificity of 99%.

Disposal

Because bacterial colonies on inoculated URICULT® Urine CULTURE-PADDLES® are actual or potential pathogens, they should not be touched and should not be unduly exposed to other office personnel or patients. It is advised that the procedure to dispose of inoculated culture media be in accordance with existing state or local laws. To avoid any risk of contamination after a culture has been interpreted, it is also recommended that used URICULT® Urine CULTURE-PADDLES® be promptly and completely immersed in a cup of bactericidal "biocide" solution such as 3% phenol, Staphene® (Vestal Labs) or Cidex® (Surgikos, Inc.).
Bactericidal-treated paddles and protective vials can then be placed in a large wide mouthed jar or other suitable disposal container filled about 1/3 of its capacity with additional biocide. Keep the container tightly capped and discard when filled.

Colony Density Chart

References

1. Mackey JP, Sandys GH: Laboratory Diagnosis of Infections of the Urinary Tract in General Practive 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.

Explanation of symbols

IVD	REF	LOT		
In vitro diagnostic medical device	Catalogue number	Batch code	Temperature limitation	Use by
Manufacturer	Consult instructions for use	Sufficient for	Protect from draught and temperature fluctuations	This way up

Contact information

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