Staphene® (Vestal Labs) or Cidex® (Surgikos, Inc.). In a cup of bactericidal solution such as 3% phenol, CULTURE-PADDLES® are actual or potential pathogens, they might cause variations in the expected bacterial colonies on the agar surface. An incubator calibrated to maintain a temperature of 97°F ± 4°F is necessary for the detection of Gram negative pathogens commonly encountered in urine specimens. The electrolyte deficient nature of the medium prevents the growth of all bacterial colonies. The inclusion of certain lactose fermenting bacteria may cause variations in the expected bacterial colonies on the agar surface. Chemical & biological principles of the procedure

CLED Agar (Chemical & biological principles of the procedure)

CLED medium was first described by Mackey and Sandys for the detection and isolation of Gram negative intestinal pathogens. The inclusion of lactose (10 g/l) allows the differentiation of lactose fermenting bacteria, since the by-products of lactose metabolism give rise to the characteristic swarming of bacteria. The inclusion of sodium chloride (3.0 g/l) offers exclusion of lactobacilli fermenting bacteria, since the byproducts of fermentation cause dehydration of the agar. The pH values of CLED medium may vary depending on the degree of metallic sheen.

Storage

● Store at 45...77°F (7...25°C) in the package provided.

Contents of the kit & typical formulation

**CLED medium**

- Media No. 1000

- 2.0 g/l Thymol

- 5.0 g/l L-Cystine

- 8.0 g/l Phosphate

- 10.0 g/l Eosin Y

- 3.0 g/l Peptone

- 8.0 g/l Meat extract

- 3.0 g/l Phosphate

- 2.0 g/l Thymol

**EMB medium**

- Media No. 1300

- 2.0 g/l Thymol

- 8.0 g/l L-Cystine

- 5.0 g/l Iodine

- 2.0 g/l Disgigalup

- 5.0 g/l Peptone

- 2.0 g/l Thymol

- 3.0 g/l Eosin Methylene Blue

- 3.0 g/l Phosphate

- 1.0 g/l Phosphate

Test procedure

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

1. Remove the URICULT® Urine CULTURE-PADDLE® from the protective cap by uncapping the cap.

2. Handling the URICULT® Urine CULTURE-PADDLE® by the handle only, pour the contents of the culture-paddle into the petri dish with approximately 3 ml urine. Place the culture-paddle in the petri dish with the agar surface down. This will allow for the detection of slow growing bacteria.

3. Allow the contents to air dry for at least 15 minutes. The agar surfaces should be fully immersed in the urine sample. If the agar surface is not fully immersed, it is possible to obtain a false negative result.

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

5. Complete plate culturing following the procedure outlined in the Certificate of Analysis.

6. Remove URICULT® vial from incubator following isolation and identification of bacteria. The culture-paddle is suspended in a clear plastic vial. Do not remove from the paddle or evidence of mold or bacterial growth. The laboratory should inspect the inoculated URICULT® Urine CULTURE-PADDLE® for any visible contamination. The lot number can be found on the protective cap by unscrewing the vial cap.

7. Inoculate a slant of the EMB medium with 0.01 ml of the culture-paddle suspension. Prepare a second slant as a control culture. Starch should be reduced in less than 24 hours.

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

Cultural & biological principles of the procedure

**URICULT® Urine CULTURE-PADDLES®**

- Culture-paddles are actual or potential pathogens, they might cause variations in the expected bacterial colonies on the agar surface. The laboratory should inspect the inoculated URICULT® Urine CULTURE-PADDLE® for any visible contamination. The lot number can be found on the protective cap by unscrewing the vial cap. If the agar surface is not fully immersed, it is possible to obtain a false negative result.

- Because bacterial colonies on inoculated URICULT® Urine CULTURE-PADDLES® are for IN VITRO diagnostic use only, a bacterial colony may be observed across the agar surface during the growth of related bacterial colonies. This lot meets the NCCLS Approved Standard for commercially prepared media. This lot meets the Quality Control Criteria set by Orion Diagnostica Oy for microbial load (contamination).

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- Culture disposal

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- Biological principles of the procedure

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Colony Density Chart

Results

Following the inoculation of an inoculated URICULT® Urine CULTURE-PADDLE®, the presence of bacterial colonies can be determined at the time of manufacture. Colony counts derived from serial dilution are performed on both sides of the URICULT® CULTURE-PADDLE® at the time of manufacture. Media wellbeing, no more than 48 hours. Evidence by URICULT® and colony morphology will result in a presumptive identification. Bacterial variation may occur in characteristic colony morphology. The Colony Density Chart allows the reporting of colony counts for each organism.

Limitations of procedure

1. Interpret the results following 24 hour incubation as follows:

- Greater than 100,000 CFU/ml urine
- 100,000 (10^5) CFU/ml, and "Confluent growth" (complete coverage of the agar surfaces) may be misinterpreted as a negative result.

2. Repeat each bacterial suspension in a separate URICULT® Urine CULTURE-PADDLE® and incubate in the manner described in the test procedure section of this manual.

3. Repeat the interpretation following 24 hour incubation as follows:

- Growth of transparent colonies with a color change toward blue on the CLED medium.
- Growth of translucent colonies with a color change toward yellow on the CLED medium.
- Growth of translucent colonies with a color change toward yellow on the EMB medium.
- Growth of pin point colonies on the EMB medium.
- Growth of yellow colonies with a color change toward yellow on the CLED medium.
- Growth of pin point colonies on the CLED medium and growth of white colonies on the EMB medium.
- Growth of pin point colonies on the CLED medium and growth of purple or metallic colonies on the EMB medium.
- No growth on both sides of the CLED and EMB media.

Performance characteristics

- This product was tested and found to meet the MCCB standards: American Society for Testing and Materials.
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Quality control

1. Prepare separate suspensions of the following organisms in sterile, water-free, 0.85% saline:

- Staphylococcus aureus ATCC 25923
- Enterococcus faecalis ATCC 29212
- Proteus vulgaris ATCC 15442
- Pseudomonas aeruginosa ATCC 27853
- Staphylococcus epidermidis ATCC 12228
- Serratia marcescens ATCC 13880
- Salmonella typhimurium ATCC 13311
- E. coli ATCC 8739

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- Staphylococcus epidermidis ATCC 12228
- Serratia marcescens ATCC 13880
- Salmonella typhimurium ATCC 13311
- E. coli ATCC 8739

3. Interpret the results following 24 hour incubation as follows:

a. The product met the performance criteria, and the incidence of laboratory contamination was less than 3.5%.

b. The product met the performance criteria, and the incidence of laboratory contamination was less than 3.5%.

4. Describe each bacterial suspension in a separate URICULT® Urine CULTURE-PADDLE® and incubate in the manner described in the test procedure section of this manual.

5. Repeat each bacterial suspension in a separate URICULT® Urine CULTURE-PADDLE® and incubate in the manner described in the test procedure section of this manual.

6. Repeat the interpretation following 24 hour incubation as follows:

- Growth of transparent colonies with a color change toward blue on the CLED medium.
- Growth of translucent colonies with a color change toward yellow on the CLED medium.
- Growth of translucent colonies with a color change toward yellow on the EMB medium.
- Growth of pin point colonies on the EMB medium.
- Growth of yellow colonies with a color change toward yellow on the CLED medium.
- Growth of pin point colonies on the CLED medium and growth of purple or metallic colonies on the EMB medium.
- No growth on both sides of the CLED and EMB media.

References

1. Mackey JP, Sandys GH: Laboratory Diagnosis of Infec