Cystine-Lactose-Electrolyte-Deficient Agar (CLED) is a differential media used for the isolation and enumeration of bacteria in urine specimens. It supports the growth of urinary pathogens and contaminants but reduces the effects of swarming Proteus species due to its lack of electrolytes.

**SUMMARY AND EXPLANATION**

The method for restricting the swarming Proteus sp. by removing electrolytes from solid culture medium was originally developed by Sandys in 1960. Various modifications of this electrolyte deficient medium were developed by Mackey and Sandys (substitution of lactose for mannitol, increased concentrations of brom thymol blue indicator and agar, and incorporation of cystine) to obtain the current CLED formulation which was reported to be ideal for dip-inoculum techniques and for urinary bacteriology in general.

**PRINCIPLES OF THE PROCEDURE**

The nutrients in CLED agar are supplied by the peptones, pancreatic digests of gelatin and casein, and beef extract. Lactose is provided as a fermentative energy source and cystine permits the growth of "dwarf colony" coliforms. Colonies which have the ability to ferment lactose will lower the pH of the medium turning the medium from green to yellow as indicated by a change in the brom thymol blue indicator.

**TYPICAL FORMULA AND APPEARANCE**

Appearance = pale green, translucent
(Approximate formula* per liter of processed water)

- Pancreatic Digest of Gelatin, 4.0g
- Pancreatic Digest of Casein, 4.0g
- Beef Extract, 3.0g
- L-Cystine, 0.128g
- Brom thymol blue, 0.02g
- Agar, 15.0g

*Adjusted and/or supplemented to meet performance criteria.

**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.

**STORAGE/SHELF LIFE**

Plated media should be stored at 2-8°C (36-46°F), media side up, in the unopened or resealed package protected from light. Do not freeze or expose to high temperature. 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.

**SPECIMEN COLLECTION**

The quality of culture results depends primarily on the adequacy and condition of the specimen submitted for examination. Proper specimen collection techniques must be followed to ensure the most accurate culture results. Specimens should be collected prior to the initiation of antimicrobial 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.

CLED Agar should be inoculated using a quantitative culture method promptly after specimen collection. If a delay in inoculation exceeding two hours in unavoidable, specimens may be stored at refrigerated temperatures (2-8°C/36-46°F) in a closed sterile container for a period not to exceed 24 hours.

Specimens may contain microorganisms that may be potentially infectious. Strict adherence to aseptic techniques and established precautions should be followed throughout the procedure.

**MATERIALS PROVIDED**

CLED Agar Plates – 10 each

**MATERIALS REQUIRED BUT NOT PROVIDED**

Incubator maintaining 33 - 37°C.
Ancillary culture media, reagents and laboratory equipment as required.

**PROCEDURE**

Remove CLED plates from the unopened or resealed refrigerated package and allow media to reach room temperature. Resuspend urine specimen by gently swirling container. Immerse an inoculating loop into the urine specimen up to the loop-shaft junction. Remove loop to obtain a sample. (Note: Ensure an intact drop of urine is contained within the loop.) Using aseptic technique, transfer the specimen on the loop to the CLED medium. Dispense the drop by touching the loop gently to the agar surface and streak down the center of the entire plate. 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.) Place the plate, media side up, into the incubator at 33 - 37°C for 18-24 hours.

**EXPECTED RESULTS**

NCCLS CONTROL ORGANISMS (ATCC STRAINS)

- *Escherichia coli* (ATCC 25922) Growth; yellow center
- *Proteus vulgaris* (ATCC 8427) Growth; bluish
- *Staphylococcus aureus* (ATCC 25923) Growth; deep yellow

**LABORATORY RESULTS**

Count the number of colonies on the medium. Multiply the result by the appropriate factor for the calibrated inoculating loop to the convert the count to colony forming units per milliliter (cfu/ml). Typical colonial morphology on CLED Agar is as follows:
Yellow colonies, opaque, center slightly deeper yellow

Yellow to whitish blue colonies, very mucoid

Translucent blue colonies

Green colonies with typical matted surface and rough edges

Small yellow colonies

Deep yellow colonies

Pale yellow opaque colonies

LIMITATIONS

The ability to detect microorganisms by culture techniques can be affected by the following factors: improper specimen collection, storage and inoculation, initiation of antimicrobial 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 organisms requires additional testing which may include: Gram stain, oxidase, catalase, and other biochemical test. Additional information on organism identification can be found in the microbiological reference materials.\(^3\)

REFERENCES


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: 33-37°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 in "STORAGE/SHELF LIFE" section.

2. Peel off the lower portion of a product bag label 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 have technical questions answered; please call between the hours of 9:00 am to 5:00 pm EST.

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