PRODUCT INFORMATION AND QUALITY CONTROL SHEET
CLED AGAR PLATE

INTENDED USE
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[1]. 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	4.0
Beef Extract	3.0
Lactose	10.0
L-Cystine	0.128
Brom thymol blue	0.02
Agar	15.0

*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^{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.

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, catheteriation, 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.[2,3]

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^{0}C/36 - 46^{0}F) in a closed sterile container for a period not to exceed 24 hours.[2,3]

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^{0}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^{0}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:

Escherichia coli	Yellow colonies, opaque, center slightly deeper yellow
Klebsiella sp.	Yellow to whitish blue colonies, very mucoid
Proteus sp.	Translucent blue colonies
Pseudomonas aeruginosa	Green colonies with typical matted surface and rough edges
Enterococci	Small yellow colonies
Staphylococcus aureus	Deep yellow colonies
Staphylococci, coagulase negative	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. [2,3]

REFERENCES

1. Sandys, G.H. 1960. A new method of preventing swarming *Proteus* species with a description of new media suitable for use in routine laboratory practice. J. Med. Lab. Technol. Vol 17:224-233.

3. Clarridge, J. E., M. T. Pezzlo, and K. L. Vost. 1987. Cumitech 2A, Laboratory Diagnosis of Urinary Tract Infection. Coord.ed., T.L. Gavan. American Society for Microbiology, Washington, D.C.

4. Lennette, E.H., ed. 1985. Manual of Clinical Microbiology 4th ed. American Society for Microbiology, Washington, D.C.

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 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.

HealthLink
3611 St. Johns Bluff Rd. So. Ste. 1
Jacksonville, FL 32224

1-800-638-2625

May, 2007

Product No. 1024 Rev. No. 02