BD™ CLED Agar

INTENDED USE

BD CLED Agar (Cystine-Lactose-Electrolyte-Deficient Agar) is a differential culture medium for use in isolating and enumerating bacteria from urine. It supports the growth of urinary pathogens and contaminants but prevents undue swarming of *Proteus* species due to its lack of electrolytes.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

In 1960, Sandys reported on the development of a new method of preventing the swarming of *Proteus* on solid media by restricting the electrolytes in the culture medium which was modified later several times for use in urine culture.[1-3] It was designated as Cystine-Lactose-Electrolyte-Deficient (CLED) medium and reported to be ideal for dip-inoculum techniques and for urinary bacteriology in general.

The nutrients in **BD CLED Agar** are supplied by the gelatin and casein peptones, and beef extract. Lactose is included to provide an energy source for organisms capable of utilizing it by a fermentative mechanism. Bromthymol blue is used as a pH indicator to differentiate lactose fermenters from lactose-nonfermenters. Organisms which ferment lactose will lower the pH and change the color of the medium from green to yellow. The cystine permits the growth of "dwarf colony" coliforms.[3] Electrolyte sources are reduced in order to minimize the swarming of *Proteus* species. Thus, the medium allows quantitative determination of urinary pathogens including *Proteus* when calibated loops are used for inoculation.

REAGENTS

BD CLED Agar

Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin	4.0 g
Pancreatic Digest of Casein	4.0
Beef Extract	3.0
Lactose	10.0
L-Cystine	0.128
Bromthymol Blue	0.02
Agar	15.0

pH 7.3 +/- 0.2

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate plates at 35 ± 2°C in an aerobic atmosphere. Examine plates at 18 to 24 h for amount of growth, pigmentation, colony size and inhibition of *Proteus* swarming/spreading.

Strains	Growth Results
Escherichia coli ATCC™ 25922	Growth; colonies yellow, medium yellow
Proteus vulgaris ATCC 8427	Growth; colonies colorless to blue; swarming inhibited; slight spreading acceptable
Enterococcus faecalis ATCC 29212	Growth; colonies colorless to yellow; medium yellow
Staphylococcus aureus ATCC 25923	Growth; colonies small, yellow; medium yellow
Staphylococcus saprophyticus ATCC 15305	Growth; colonies small, white to yellowish; medium yellow
Uninoculated	Green to blue-green

PROCEDURE

Materials Provided

BD CLED Agar (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types and Collection of Specimens

This medium is exclusively used for enumerating and differentiating bacteria in urine. Midstream or catheter urine, or urine collected by suprapubic bladder puncture can be used (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). Observe aseptic techniques for collecting urine specimens. Urine must be directly streaked onto the medium not later than 2 hours after collection or must be kept refrigerated (not longer than 24 hours) to avoid overgrowth of the infectious agents or contaminants before inoculation of this medium.[2-5]

Test Procedure

Collect a sample of the undiluted, well-mixed urine using a calibrated loop (0.01 or 0.001 ml). Ensure proper loading of the loop with the specimen. Inoculate the sample down the middle of the plate in a single streak from which additional spreading of the inoculum is performed. [4,5] Incubate plates in ambient air at 35 ± 2°C for 24 to 48 h.

Results

Typical colonial morphology on **BD CLED Agar** is as follows:

Organisms	Growth Results
Escherichia coli	Yellow colonies, opaque; yellow medium
Klebsiella, Enterobacter	Yellow to whitish-blue colonies, often mucoid; yellowish medium
Proteus	Translucent blue colonies; blue-green to blue medium
Pseudomonas aeruginosa	Green colonies with typical matted surface and rough periphery; blue medium
Enterococci	Small yellow colonies, about 0.5 mm in diameter; yellow medium
Staphylococcus aureus	Deep yellow colonies, uniform in color; yellow medium
Coagulase negative staphylococci	Pale yellow colonies, more opaque than *Enterococcus faecalis*

Calculation and Interpretation of Results

Count the number of colonies (CFU) on the plate. If a 0.01 ml loop was used, each resultant colony is representative of 100 CFU/ml; if a 0.001 ml loop was used, each colony corresponds to 1000 CFU/ml of urine.[5]

Midstream and catheter urine: Current guidelines indicate that for a single isolate a density of $\geq 10^5$ CFU/ml indicates infection, $<10^5$ CFU/ml indicates urethral or vaginal contamination, and between 10^4 to 10^5 CFU/ml needs to be re-evaluated based on clinical information.[6]

Contaminant bacteria usually appear in low numbers which vary in colonial morphology.

Urine collected by suprapubic bladder puncture: Since the bladder is sterile in non-infected individuals, any CFU detected indicates an infection.

Urinary pathogens will usually yield high counts having uniform colonial morphology and must be subcultured directly to routine media for identification and susceptibility testing.[5,6]

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD CLED Agar is suitable for the isolation and counting of many aerobically growing microorganisms, such as *Enterobacteriaceae*, *Pseudomonas* and other non-fermenting Gram negative rods, enterococci, staphylococci, *Candida* species, and many others from urine specimens.

Streptococci and other organisms requiring blood or serum for growth may only be insufficiently recovered on this medium or may need extended incubation. Therefore, the specimen should also be cultivated onto a blood agar plate if such organisms are expected.
Genitourinary pathogens such as *Neisseria gonorrhoeae, Gardnerella vaginalis, Chlamydia, Ureaplasma,* or other fastidious organisms do not grow on this medium. Consult the references for the appropriate detection techniques of these organisms.[4-6]
Although a differentiation according to lactose fermentation and certain other diagnostic tests may be performed directly on this medium, biochemical and, if indicated, serological testing using pure cultures is necessary for complete identification.

REFERENCES

1. Sandys, G.H. 1960. A new method of preventing swarming of *Proteus* sp. with a description of a new medium suitable for use in routine laboratory practice. J. Med. Lab. Technol. 17:224-233.
2. Mackey, J.P., and G.H. Sandys. 1965. Laboratory diagnosis of infection of the urinary tract in general practice by means of a dip-inoculum transport medium. Br. Med. J. 2:1286-1288.
3. Mackey, J.P., and G.H. Sandys. 1966. Diagnosis of urinary infections. Br. Med. J. 1:1173.
4. Barry, A.L., P.B. Smith, and M. Turck. 1975. Cumitech 2, Laboratory diagnosis of urinary tract infections. Coordinating ed., T.L. Gavan. American Society for Microbiology, Washington, D.C.
5. Thomson, R.B., and J.M. Miller. 2003. Specimen collection, transport, and processing: bacteriology. *In:* Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
6. Clarridge, J.E., M.T. Pezzlo, and K.L. Vosti. 1987. Cumitech 2A, Laboratory diagnosis of urinary tract infections. Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

PACKAGING/AVAILABILITY

BD CLED Agar

Cat. No. 254003	Ready-to-use plated media, 20 plates
Cat. No. 254070	Ready-to-use plated media, 120 plates

FURTHER INFORMATION

For further information please contact your local BD representative.

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