CLED Agar

Intended Use

CLED Agar is used for the isolation, enumeration and presumptive identification of microorganisms from urine.

Summary and Explanation

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.[1] Previous chemical methods used to inhibit swarming by *Proteus* included the addition of chloral hydrate, alcohol, sodium azide, surface-active agents, boric acid and sulfonamides to the culture medium.[1]

This electrolyte-deficient medium of Sandys was modified by Mackey and Sandys[2] for use in urine culture by substituting lactose and sucrose for the mannitol and increasing the concentrations of the bromthymol blue indicator and of the agar. These two investigators further modified the medium by the incorporation of cystine in order to enhance the growth of cystine-dependent "dwarf colony" coliforms and by deletion of sucrose.[3] They designated the new medium as Cystine-Lactose-Electrolyte-Deficient (CLED) medium and reported it to be ideal for dip-inoculum techniques and for urinary bacteriology in general.

CLED Agar is recommended for use in plates or in urine dipsticks for detecting significant bacteriuria by quantitative culture of urine. For reliable results, inoculation of the medium must occur as soon after collection as possible. Confluent or semiconfluent growth of bacteria will occur on the surface of the dipstick medium when bacterial counts are greater than 10^5 per mL of urine, as confirmed by plates inoculated by the calibrated-loop or duplicate-dilution pour-plate methods.[4] Once the medium has been inoculated by immersion of the dipstick or by pouring the urine over the surface of the medium if only a small volume is available, the dipstick may be held 48 hours or longer, refrigerated or at room temperature until received in the laboratory. On receipt, the dipstick should be incubated at 35 ± 2°C for 18-24 hours, to allow colonies to develop on the medium.

Principles of the Procedure

The nutrients in CLED Agar are supplied by peptones, pancreatic digests of gelatin and casein, and beef extract. Lactose is included to provide an energy source for organisms capable of utilizing it by a fermentative mechanism. The cystine permits the growth of "dwarf colony" coliforms. Bromthymol blue is used as a pH

User Quality Control

Identity Specifications

BBL™ CLED Agar

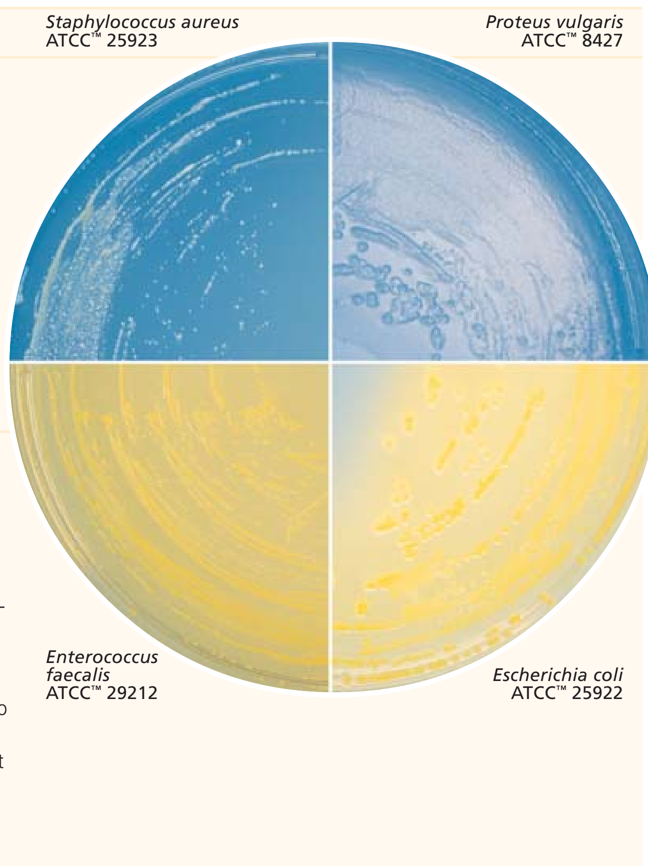
Dehydrated Appearance:	Fine, homogenous, free of extraneous material.
Solution:	3.6% solution, soluble in purified water upon boiling. Solution is medium, yellow green to blue green, clear to slightly hazy, with up to a large amount of minute suspended insolubles.
Prepared Appearance:	Medium, yellow green to blue green, clear to slightly hazy.
Reaction of 3.6% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ CLED Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 42-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	REACTION
Enterococcus faecalis	29212	10^3-10^4	Good	Yellow
Escherichia coli	25922	10^3-10^4	Good	Yellow
Klebsiella pneumoniae	33495	10^3-10^4	Good	With or without green to yellow reaction
Pseudomonas aeruginosa	10145	10^3-10^4	Good	With or without blue reaction
Staphylococcus aureus	25923	10^3-10^4	Good	Yellow
Proteus vulgaris	8427	10^3-10^4	Good	Blue



indicator to differentiate lactose fermenters from lactose nonfermenters. Organisms that ferment lactose will lower the pH and change the color of the medium from green to yellow. Electrolyte sources are reduced in order to restrict the swarming of *Proteus* species.

Bacteriuria is determined by inoculating the surface of an agar medium using 0.1 mL of a 10^{-2} dilution of the urine sample or using a calibrated loop (0.001 mL) of the undiluted sample.[5] Current guidelines are that for a single isolate a density of $>10^5$ CFU/mL indicates infection, $<10^4$ CFU/mL indicates urethral or vaginal contamination, and between 10^4 and 10^5 CFU/mL needs to be evaluated based on clinical information.[6]

Formula

BBL™ CLED Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Gelatin	4.0	g
Pancreatic Digest of Casein	4.0	g
Beef Extract	3.0	g
Lactose	10.0	g
L-Cystine	128.0	mg
Bromthymol Blue	0.02	g
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 36 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the medium as soon as possible after the specimen is received in the laboratory. It is recommended that quantitative methods be used for culturing urine specimens.[5] Incubate at 35 ± 2°C for 24-48 hours.

Expected Results

Count the number of colonies on the plate or dipstick. Multiply by an appropriate number to convert the count to CFU per mL of sample.

Contaminant bacteria usually appear in low numbers which vary in colonial morphology. Urinary pathogens will usually yield high counts having uniform colonial morphology and should be subcultured directly to routine media for identification and susceptibility testing.[5,7]

Typical colonial morphology on CLED Agar is as follows:

Organism	Colonial morphology
Escherichia coli	Yellow colonies, opaque, center slightly deeper yellow
Klebsiella	Yellow to whitish-blue colonies, extremely mucoid
Proteus	Translucent blue colonies
Pseudomonas aeruginosa	Green colonies with typical matted surface and rough periphery
Enterococci	Small yellow colonies, about 0.5 mm in diameter
Staphylococcus aureus	Deep yellow colonies, uniform in color
Staphylococci coagulase-negative	Pale yellow colonies, more opaque than *E. faecalis*

Limitations of the Procedure

Factors that may cause urine counts from infected patients to be low include: rapid rate of urine flow, prior initiation of antimicrobial therapy, a urine pH of less than 5 and a specific gravity of less than 1.003.[7]

References

1. Sandys. 1960. J. Med. Lab. Technol. *17*:224.
2. Mackey and Sandys. 1965. Br. Med. J. *2*:1286.
3. Mackey and Sandys. 1966. Br. Med. J. *1*:1173.
4. Benner. 1970. Appl. Microbiol. *19*:409.
5. Barry, Smith and Turck. 1975. Cumitech 2, Laboratory diagnosis of urinary tract infections. Coord. ed., Gavan. American Society for Microbiology, Washington, D.C.
6. Clarridge, Pezzlo and Vosti. 1987. Cumitech 2A, Laboratory diagnosis of urinary tract infections. Coordinating ed., Weissfeld. American Society for Microbiology, Washington, D.C.
7. Finegold and Martin. 1982. Bailey & Scott's diagnostic microbiology, 6th ed. The C.V. Mosby Company, St. Louis, Mo.

Availability

BBL™ CLED Agar

Cat. No. 212218 Dehydrated – 500 g

United States and Canada

Cat. No. 221850 Prepared Plates – Pkg. of 10*
221530 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 254003 Prepared Plates – Pkg. of 20*
254070 Prepared Plates – Ctn. of 120*

Japan

Cat. No. 251953 Prepared Plates – Pkg. of 20*
251530 Prepared Plates – Ctn. of 100*

**Store at 2-8°C.*