C.L.E.D. Agar with Bromothymol Blue

Intended Use
C.L.E.D. Agar with Bromothymol Blue is recommended for isolation, enumeration and presumptive identification of urinary pathogens on the basis of lactose fermentation.

Summary
Sandys observed that restricting the electrolytes on a solid medium might prevent the swarming of *Proteus*. Previous chemical methods used to inhibit swarming of *Proteus* included the addition of chloral hydrate, alcohol, sodium azide, surface-active agents, boric acid and sulphonamides to the culture medium. This electrolyte medium was modified for use in urine culture by substituting lactose and sucrose instead of mannitol and increasing the concentrations of bromothymol blue indicator and agar. The medium was further modified by the incorporation of cystine in order to enhance the growth of cystine-dependent "dwarf colony" coliforms and by the deletion of sucrose. This new medium, Cystine-Lactose-Electrolyte-Deficient (C.L.E.D.) Agar is ideal for dip-inoculation techniques and for urinary bacteriology in general.

Principle
The essential growth nutrients are supplied by Pancreatic digest of gelatin, Pancreatic digest of casein and beef extract. Lactose is the carbohydrate source. L-cystine permits the growth of "dwarf colony" coliforms. Bromothymol blue is used as the pH indicator to differentiate lactose fermenters from non-lactose fermenters. Organisms that ferment lactose will lower the pH and change the colour of the medium from green to yellow. Electrolyte sources are reduced in order to restrict the swarming of *Proteus* species.

Formula*

Ingredients	**g/L**
Pancreatic digest of gelatin	4.0
Pancreatic digest of casein	4.0
Lactose	10.0
Beef Extract	3.0
L-Cystine	0.128
Bromothymol Blue	0.02
Agar	15.0
Final pH (at 25°C)	7.3 ± 0.2

*Adjusted to suit performance parameters

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2ºC-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Clinical samples – urine

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend 36.15 g of the powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.

Quality Control

Dehydrated Appearance: Greenish yellow coloured, homogenous, free flowing powder.
Prepared Appearance: Green to blue green coloured, clear to slightly opalescent gel forms in petridishes.
Cultural Response: Cultural characteristics observed after an incubation of 42-48 hours at 35°C -37°C.

Organism (ATCC)	Growth	Colour of Colony
Staphylococcus aureus subsp. *aureus* (25923)	Good	Deep yellow
Escherichia coli (25922)	Good	Yellow, opaque, center slightly deeper yellow
Enterococcus faecalis (29212)	Good	Yellow
Proteus mirabilis (25933)	Good	Blue
Klebsiella pneumoniae subsp. *pneumoniae* (10031)	Good	Yellow, mucoid

Interpretation of Results

1. Count the number of colonies on the dipstick. Multiply by dilution factor to convert the count to CFU per mL of the sample.
2. Contaminant bacteria usually appear in low numbers, which vary in colony morphology.
3. Typical colony morphology on C.L.E.D. Agar is as follows:

E. coli	Yellow colonies, opaque, center slightly deeper yellow.
Klebsiella	Yellow to whitish-blue colonies, extremely mucoid.
P. aeruginosa	Green colonies with typical matted surface and rough periphery.
Enterococci	Small yellow colonies, about 0.5 mm in diameter.
S. aureus	Deep yellow colonies, uniform in colour.
Proteus	Translucent blue colonies
Coagulase negative Staphylococci	Pale yellow colonies, more opaque than *E. faecalis.*

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. 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.
2. *Shigella* species may not grow on this medium.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

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. Dixson J. M. S. and Clark M. A., 1968, Conc. Med. Assoc. J., 99 (15)
5. Benner E. J., 1970, Appl. Microbiol., 19(3), 409
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
7. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201030100100	Dehydrated Culture Media	100 g
201030100500	Dehydrated Culture Media	500 g
201030102500	Dehydrated Culture Media	2.5 k
205030380100	Ready Prepared Plate (90 mm)	100 Plates
205030380020	Ready Prepared Plate (90 mm)	20 Plates
201030092500	Dehydrate Culture Media	2.5 k
203030270100	Bottle Media	100 mL