

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

Intended Use

C.L.E.D. Agar with Bromothymol Blue Plate is used for isolation and differentiation of urinary tract pathogens on the basis of lactose fermentation.

Summary

Sandy's 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
Beef Extract	3.0
Lactose	10.0
L-cystine	128.0
Bromothymol Blue	0.02
Bacteriological Agar	15.0

*Adjusted to suit performance parameters.

Additional Material Required

Bacteriological Incubator.

Instructions for use

1. Open the sterile pack and remove the plates aseptically.
2. Inoculate the plate immediately after urine collection as per standard procedure.
3. Incubate the plates in inverted position at appropriate temperature and time.

Reading and interpretation

1. After incubation, observe the microbial growth and count the colonies.
2. Interpretation is assured by user.

Quality Control

Appearance: Gel with smooth and even surface, without any cracks, bubbles and drying or shrinking of media.

Colour of Medium: Blue green slightly hazy.

Quantity of Medium: 26 ± 2 g in 90 mm petriplate.

pH at 25°C ± 2°C: 7.3 ± 0.2

Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP and growth is observed after an incubation at 30°C-35°C for 42-48 hours.

Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time, inoculating ≤100 cfu of appropriate microorganism.

Indicative Properties: The test results observed are within the specified temperature and time, inoculating ≤ 100 cfu of appropriate microorganism.

Growth Promoting + Indicative

Organism (ATCC)	Growth	Color of Colony
<i>Escherichia coli</i> (25922)	Good	Yellow
<i>Enterococcus faecalis</i> (29212)	Good	Greenish
<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> (10031)	Good	Yellow
<i>Pseudomonas aeruginosa</i> Strain Boston 41501 (27853)	Good	Blue
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> (25923)	Good	Yellow
<i>Proteus mirabilis</i> (25933)	Good	Blue

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.

Storage and Shelf Life

1. Store between 2°C-8°C to avoid water condensation. Condensation can be prevented by avoiding quick temperature shifts and mechanical stress.
2. Under optimal conditions, the medium has a shelf life of 3 months. Use before expiry mentioned on the label.

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. Dixon 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	Pack Size
205030380100	C.L.E.D Agar with Bromothymol Blue Plate	100 Plates
205030380020	C.L.E.D Agar with Bromothymol Blue Plate	20 Plates

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.
