C.L.E.D. Agar Base w/o Indicator

M1146

C.L.E.D. Agar w/o Indicator (with added Bromo Thymol Blue) is recommended for isolation, enumeration and presumptive identification of bacterial flora in the urinary tract.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	4.000
Casein enzymic hydrolysate	4.000
Beef extract	3.000
Lactose	10.000
L-Cystine	0.128
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36.1 grams in 998 ml distilled water. Add rehydrated contents of 1 vial of Bromo Thymol Blue Supplement (FD091). Heat, to boiling, to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

On a solid medium, Sandys reported that swarming of *Proteus* species could be controlled by restricting the electrolytes (1). Formerly swarming of *Proteus* was controlled by adding alcohol, surface-active agent, sodium azide, boric acid etc. to the medium (1). Later on Sandys medium was modified by Mackey and Sandys (2), by replacing mannitol by lactose and sucrose and elevating concentration of agar and bromo thymol blue. This formulation was further modified by the same authors and called C.L.E.D. (Cystine-Lactose-Electrolyte-Deficient) by deleting the sucrose and by including L-cystine for promoting the growth of cystine dependant dwarf coliform colony (3). This medium is recommended for use in urine bacteriology, promoting the growth of all urinary pathogens. C.L.E.D. Medium is also recommended for dipstick procedures and as dip inoculum transport medium for urine specimens (2, 3, 4).

Peptic digest of animal tissue, beef extract, casein enzymic hydrolysate provides essential growth nutrients. Lactose is the fermentable sugar. L-cystine supports the growth of dwarf coliform colony. Bromo thymol blue is the pH indicator which turns yellow at acidic pH.

Bacteriuria may be quantitated by inoculating the surface of an agar medium by proper dilution. Inoculate the medium immediately after urine collection. It can also be inoculated by calibrated loop or duplicate dilution pour plate methods (5, 6). *Shigella* species may not grow on this medium. Initiation of antibiotic therapy, before collection sample, low urine pH (less than 5) etc. may result in low bacterial count from infected patients.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
With addition of Bromo Thymol Blue Supplement (FD091): Green coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.61% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

M1146: Cultural characteristics observed with added Bromothymol Blue Supplement(FD091),after an incubation at 35-37ºC for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Cultural Response				
Escherichia coli ATCC 25922	50-100	good-luxuriant	>=70%	yellow, opaque, center slightly deeper yellow
Enterococcus faecalis ATCC 29212		good-luxuriant	>=70%	slight yellowish or greenish
Klebsiella pneumoniae ATCC 13883	50-100	good-luxuriant	>=70%	yellow to whitish blue
Proteus vulgaris ATCC 13315	50-100	good-luxuriant	>=70%	blue
Staphylococcus aureus ATCC 25923	50-100	good-luxuriant	>=70%	deep yellow
Salmonella Typhi ATCC 6539	50-100	good-luxuriant	>=70%	bluish

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date 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. 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

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