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Registered Off. : 101-A, Deep Enclave, Pocket "D", Ashok Vihar, Phase-III, Delhi-110 052 (India)
Corporate Off. : 152, Vardhman City Centre, Near Shakti Nagar Underbridge, Delhi-110052 (India)
Works : Plot No.1022, Modern Industrial Estate, Bahadurgarh-124507, Haryana (India)
Telephone : 0091 - 11 - 23646422 E-mail : tecpharm@gmail.com
Website : www.technopharmchem.com

C.L.E.D. Agar w/Bromo Thymol Blue

Product Code: CM0505
C.L.E.D. Agar w/ Bromo Thymol Blue is recommended for isolation, enumeration and identification of urinary pathogens on the basis of lactose fermentation.

Composition**

Ingredients	Gms/Litre
Peptone	4.000
Tryptone	4.000
Peptone B	3.000
Lactose	10.000
L-Cystine	0.128
Bromothymol blue	0.020
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 36.15 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle & Interpretation
On a solid medium, Sandys reported that swarming of Proteus species can be controlled by restricting the electrolytes. Formerly swarming of Proteus was controlled by adding alcohol, surface-active agent, sodium azide, boric acid etc. to the medium. Later on Sandys medium was modified by Mackey and Sandys, 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, 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 colony coliforms. This medium is recommended for use in urinary bacteriology, promoting the growth of all urinary pathogens. C.L.E.D. Medium is also recommended for dip stick procedures and as dip inoculum transport medium for urine specimens.
Peptone, Tryptone and Peptone B provides nitrogeneous and carbonaceous compounds, long chain amino acids, vitamins and other 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.

Quality Control

Appearance	:	Cream to yellow homogeneous free flowing powder.
Gelling	:	Firm, comparable with 1.5% Agar gel
Color and clarity of prepared medium	:	Green colored, 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

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Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours

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

Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Limitations
1. Initiation of antibiotic therapy, before collection of sample, low urine pH (less than 5) etc. may result in low bacterial count from infected patients.
2. Shigella species may not grow on this medium.