Technical Information

C.L.E.D. Agar (with Bromo Thymol Blue)

Product Code: DM 1792

Application: - C.L.E.D. Agar with Bromo Thymol Blue is recommended for isolation, enumeration and identification of pathogen from tract infection urinary on the basis of lactose fermentation.

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
Bromothymol blue	0.020
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

On a solid medium, Sandys reported that swarming of *Proteus* species can be checked by restricting the electrolytes [1] Which was earlier controlled by adding alcohol, surface-active agent, sodium azide, boric acid etc. to the medium [1]. Later this 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 named as C.L.E.D. (Cystine-Lactose-Electrolyte-Deficient) by replacing the sucrose and by including L-cystine for promoting the growth of cystine dependant dwarf colony coliforms[3]. 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 [2-4].

Peptic digest of animal tissue, beef extract, casein enzymic hydrolysate provide 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 using proper dilution and or by calibrated loop [5-6]. Inoculate the medium immediately after urine collection. *Shigella* species may not grow on this medium. Initiation of antibiotic therapy, before collection of sample, low urine pH (less than 5) etc. may result in low bacterial count from patients with uninary tact infection.

Methodology

Suspend 36.15 grams of powder media in 1000 ml distilled water. Shake it well & heat 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.

Quality Control

Physical Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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 range 7.10-7.50

Cultural Response/ characteristices

DM 1772: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour 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 pneumoniae 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 ATCC 25923	50-100	good-luxuriant	>=70%	deep yellow

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^{o} in sealable plastic bags for 2-5 days.

Further Reading

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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