Technical Information

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

Product Code: DM 2146

Application: - 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

Principle & Interpretation

Sandys reported that swarming of *Proteus* species on solid media could be controlled by restricting the use of electrolytes [(1)]. Formerly swarming of *Proteus* was controlled by adding alcohol, surface-active agent, sodium azide, boric acid etc. to the medium [(1)]. Later Sandys medium was modified by Mackey and Sandys [(2)], by replacing mannitol by lactose and sucrose and increasing 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 [(2-4)].

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.

Methodology

Suspend 36.1 grams of media powder in 998 ml distilled water. Add rehydrated contents of 1 vial of Bromo Thymol Blue Supplement (MS2091). Shake well and 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

With addition of Bromo Thymol Blue Supplement (MS2091): 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 2146: Cultural characteristics observed with added Bromothymol Blue Supplement (MS2091), after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli ATCC 25922	50-100	good-luxuriant	>=70%	yellow, opaque,crnter slightly deeper yellow
Enterococcus faecalis A TCC 29212	50-100	good-luxuriant	>=70%	slightly 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

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8° 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

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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