C.L.E.D HiCynth™ Agar w/ BTB

MCD792

C.L.E.D. HiCynth™ Agar w/ BTB is recommended for isolation and differentiation of urinary pathogens on the basis of lactose fermentation.

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No.1*	11.000
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

*Chemically defined peptone

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 And Interpretation

On a solid medium, Sandys reported that swarming of *Proteus* species can 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, 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 (3).C.L.E.D. HiCynth™ Agar is modified by using chemically defined peptones replacing animal or vegetable peptones to avoid BSE/TSE risks associated with animal peptones.. 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, 3, 4).

HiCynth™ peptone No.1 provide nitrogen and carbon compounds, vitamins, long chain amino acids 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 (5, 6).

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 infected patients.

Quality Control

Appearance

Cream to yellow may have slight green tinge 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

7.10-7.50

Cultural Response

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

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Cultural Response				
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

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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