C.L.E.D HiCynth™ Agar w/ Andrade Indicator

MCD352

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

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No.3*	11.000
Lactose	10.000
L-Cystine	0.128
Bromothymol blue	0.020
Andrade indicator	0.100
Agar	15.000
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

*- Chemically defined peptone

Directions

Suspend 36.25 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

Sandys reported a new technique where the swarming of *Proteus* on an agar medium could be prevented by restricting the electrolyte content in the culture medium (1). Sandys Medium was modified by Mackey and Sandys (2), by replacing mannitol with lactose and sucrose and elevating the concentration of agar and bromothymol blue. The same authors further modified this medium by retaining the lactose (deleting sucrose) and by including L-cystine for promoting the growth of cystine-dependent dwarf coliform colony (3). This later modified medium was designated as C.L.E.D. (Cystine- Lactose-Electrolyte-Deficient) Medium. 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).

C.L.E.D. Medium was further modified by Bevis (5) by incorporation of Andrades indicator. C.L.E.D. HiCynth™ Agar w/ Andrade Indicator is the modification of C.L.E.D. Agar w/ Andrade Indicator using chemically defined peptone free from animal and vegetable peptones, to avoid BSE/TSE risks associated with animal peptones. This medium provides sharper differentiation between lactose-fermenters (LF) and lactose-non-fermenters (NLF) (5). Addition of Andrades indicator enhances the appearance of colony and aids in the identification of microorganisms.

At different pH values, the colour of the medium varies from the standard medium, which is well documented by Bevis (5).

pH	Colour of C.L.E.D. medium
7.4	deep blue
7.0	bluish grey
6.8	pale grey
6.6	pinkish grey
6.4	bright red with whitish tinge
6.0	bright red

For better results, the medium should not be incubated for more than 24 hours because if lactose fermenters predominate, the entire medium may turn pink masking the presence of non-lactose fermenters. Inoculate the medium immediately after urine collection. *Shigella* species may not grow on this medium. Prior initiation of antibiotic therapy, low urine pH (less than 5) etc. may result in low urine count from infected patients.

Quality Control

Appearance
Light yellow to greyish yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Greenish blue clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.62% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH
7.30-7.70

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				
Enterobacter aerogenes ATCC 13048	50-100	good-luxuriant	>=70%	greyish green, mucoid
Enterococcus faecalis ATCC 29212	50-100	good-luxuriant	>=70%	orange-yellow or greenish
Escherichia coli ATCC 25922	50-100	good-luxuriant	>=70%	bright pink with pink halo
Proteus mirabilis ATCC 25933	50-100	good-luxuriant	>=70%	blue-green
Staphylococcus aureus ATCC 25923	50-100	good-luxuriant	>=70%	pale pink to golden-yellow
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant	>=70%	greyish green

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. Bevis T. D., 1968, J. Med. Lab. Technol., 25:38.

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