Technical Information

C.L.E.D.Medium (with Andrade Indicator)

Product Code: DM 1352

Application: - C.L.E.D. Medium w/Andrade Indicator is recommended for isolation and differentiation of urinary pathogens on the basis of lactose fermentation.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	4.000
Beef extract	3.000
Casein enzymic hydrolysate	4.000
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

Principle & 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)]. This Medium was for the modified by Mackey and Sandys[(2)], by replacing mannitol with lactose and sucrose and increasing 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)] which 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 [(2-4)].

C.L.E.D. Medium was further modified by Bevis [(5)] by adding of Andrades indicator. This medium provides clear cut 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 7.4	Colour of C.L.E.D. medium 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 there by masking the presence of non-lactose fermenters. Inoculate the medium immediately after collection of urine samples. 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.

Methodology

Suspend 36.25 grams of powder media in 1000 ml of distilled water. 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

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 range 7.30-7.70

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
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%	golden-yellow
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant	>=70%	greyish green

M352: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours

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

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