C.L.E.D. Agar w/Bromo Thymol Blue

M792

Intended use

Recommended for isolation, enumeration and identification of urinary pathogens on the basis of lactose fermentation.

Composition**

Ingredients	g / L
Peptone	4.000
Tryptone	4.000
HM Peptone B#	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

#- Equivalnet to Beef extract

Directions

Suspend 36.15 grams in 1000 ml purified/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 Sandy's medium was modified by Mackey and Sandys (2), by replacing mannitol by lactose and sucrose and elevating concentration of agar and bromothymol 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 dependent 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 (2,3,4).

Peptone, Tryptone and HM Peptone B provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins 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).

Type of specimen

Clinical samples - urine

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Initiation of antibiotic therapy, before collection of sample, low urine pH (less than 5) etc. may result in low bacterial count from infected patients.
2. *Shigella* species may not grow on this medium.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

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

7.10-7.50

Cultural Response

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 (00087*)	50-100	good-luxuriant	>=70%	slight yellowish or greenish
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=70%	yellow, opaque, centre slightly deeper yellow
Klebsiella pneumoniae ATCC 13883 (00097*)	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 subsp. *aureus* ATCC 25923(00034*)	50-100	good-luxuriant	>=70%	deep yellow

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

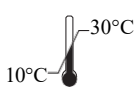
Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

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
7.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
8.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

	HiMedia Laboratories Pvt. Limited, Plot No.C-40, Road No.21Y, MIDC, Wagle Industrial Area, Thane (W) -400604, MS, India	IVD	***In vitro* diagnostic medical device**		**Storage temperature**
EC REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn, NL www.cepartner4u.eu	CE	**CE Marking**		**Do not use if package is damaged**

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