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

MCD352

Intended Use:

Recommended for isolation and differentiation of urinary pathogens on the basis of lactose fermentation.

Composition**

Ingredients	g / L
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 gram in 1000 ml of 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

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 the same 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.

Type of specimen

Clinical:Urine sample

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. This medium is recommended for urine infection. Low urine count may be a result of antibiotic therapy, low pH of urine.
2. Recovery depends on the urine count.
3. Inoculate the medium immediately after urine collection.
4. *Shigella* species may not grow on this medium.
5. 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.

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

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	good-luxuriant	>=70%	greyish green
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=70%	bright pink with pink halo
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant	>=70%	orange-yellow or greenish
Proteus mirabilis ATCC 25933	50-100	good-luxuriant	>=70%	blue-green
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	good-luxuriant	>=70%	golden-yellow
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant	>=70%	greyish green

Key : (*) Corresponding WDCM numbers.

(#) Formerly known as *Enterobacter aerogenes*

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

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.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. 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.

Revision : 02 2024