# TM 054 – C.L.E.D. AGAR W/ ANDRADE INDICATOR (CYSTINE LACTOSE ELECTROLYTE DEFICIENT AGAR)

## INTENDED USE

For isolation and differentiation of microorganisms based on lactose fermentation.

## PRODUCT SUMMARY AND EXPLANATION

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. Sandys Medium was modified by Mackey and Sandys, 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. This later modified medium was designated as C.L.E.D. (Cystine-LactoseElectrolyte-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. C.L.E.D. Medium was further modified by Bevis by incorporation of Andrades indicator. This medium provides sharper differentiation between lactose-fermenters (LF) and lactose-non-fermenters (NLF). Addition of Andrades indicator enhances the appearance of colony and aids in the identification of microorganisms.

## COMPOSITION

| Ingredients | Gms / Ltr |
|---|---|
| **Peptone** | 4.000 |
| **Beef extract** | 3.000 |
| **Tryptone** | 4.000 |
| **Lactose** | 10.000 |
| **L-Cystine** | 0.128 |
| **Bromothymol blue** | 0.020 |
| **Andrade indicator** | 0.100 |
| **Agar** | 15.000 |

## PRINCIPLE

The essential nutrients are supplied by peptone, tryptone and beef extract. Lactose is the carbohydrate source. L-cystine permits the growth of "dwarf colony" coliforms. Addition of Andrade indicator 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.

## INSTRUCTION FOR USE

- Dissolve 36.25 grams in 1000 ml of purified/distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Cool to 45-50°C.
- Mix well and pour into sterile Petri plates.

## QUALITY CONTROL SPECIFICATIONS

**Appearance of Powder** : Light yellow to greyish yellow homogeneous free flowing powder.
**Appearance of prepared medium** : Greenish blue clear to slightly opalescent gel forms in Petri plates.
**pH (at 25°C)** : 7.5±0.2

## INTERPRETATION

Cultural characteristics observed after incubation.

| Microorganism | ATCC | Inoculum (CFU/ml) | Growth | Recovery | Colour of colony | Incubation Temperature | Incubation Period |
|---|---|---|---|---|---|---|---|
| *Klebsiella aerogenes* | 13048 | 50-100 | Good-luxuriant | >=50% | Greyish green, mucoid | 35-37°C | 18-24 Hours |
| *Escherichia coli* | 25922 | 50-100 | Good-luxuriant | >=50% | Bright pink with pink halo | 35-37°C | 18-24 Hours |
| *Enterococcus faecalis* | 29212 | 50-100 | Good-luxuriant | >=50% | Orange-yellow or greenish | 35-37°C | 18-24 Hours |
| *Proteus mirabilis* | 25933 | 50-100 | Good-luxuriant | >=50% | Blue-green | 35-37°C | 18-24 Hours |
| *Staphylococcus aureus subsp. aureus* | 25923 | 50-100 | Good-luxuriant | >=50% | Golden-yellow | 35-37°C | 18-24 Hours |
| *Streptococcus pyogenes* | 19615 | 50-100 | Good-luxuriant | >=50% | Greyish-green | 35-37°C | 18-24 Hours |

## PACKAGING:

In pack size of 100 gm and 500 gm bottles.

## STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
**Product Deterioration:** Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

## DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

## REFERENCES

1. Bevis T. D., 1968, J. Med. Lab. Technol., 25:38.
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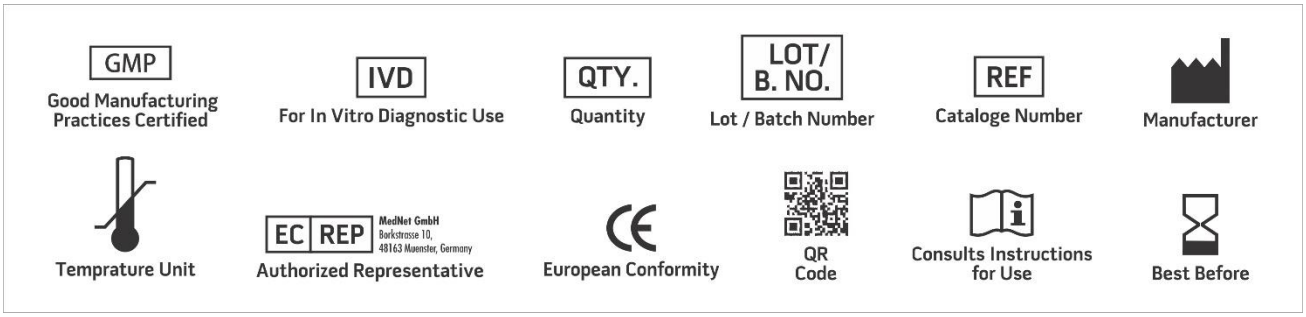
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1

5. Mackey and Sandys, 1965, Br. Med. J., 2:1286.
6. Mackey and Sandys, 1966, Br. Med. J., 1:1173.
7. Sandys, 1960, J. Med. Lab. Technol., 17:224.




**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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