

C.L.E.D.

REF 63794

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CYSTINE LACTOSE ELECTROLYTE DEFICIENT AGAR FOR THE ISOLATION AND DIFFERENTIATION OF MICROORGANISMS ISOLATED FROM URINE



2014/04

1- INTENDED USE

Cystine Lactose Electrolyte Deficient (C.L.E.D.) agar is a medium enabling the isolation, enumeration and differentiation of microorganisms isolated from urine.

2- PRINCIPLE

Differentiation of bacteria is based on their ability to ferment lactose. Fermentation of lactose induces acidification, causing the formation of **yellow** colonies in the presence of bromothymol blue (pH indicator).

The low electrolyte content inhibits invasion by *Proteus*.

3- HOW SUPPLIED

- Ready to use medium:
 - Box of 20 Petri dishes (90 mm) (CLED) code 63794
- Dehydrated medium
 - Bottle of 500g code 64364

4- THEORETICAL COMPOSITION (g/l of distilled water)

C.L.E.D. medium is prepared according to the formula described by Sandys G.H. (1) and modified by Mackey J.P. *et al.* (2)

Peptone	4
Meat extract	3
Meat peptic digest	4
L. cystine	0.128
Lactose	10
Bromothymol blue	0.02
Agar	13
Final pH	7.3 ± 0.2

Preparation of the medium:

Homogenize the powder contained in the bottle.

Mix **34.2 grams** of dehydrated medium to one litre of sterile distilled water until a homogeneous suspension is obtained.

Heat gently, shaking frequently, then heat to boiling until complete dissolution.

Sterilize at 121°C for 15 minutes. Dispense into Petri dishes or bottles.

5- STORAGE

- Ready to use medium: at +2-8°C.
- Dehydrated medium: tightly sealed bottle in a dry place at +15-25°C

The expiry date and batch number are indicated on the packaging.

6- INSTRUCTIONS

Material:

- Material provided: C.L.E.D. medium.

Inoculation:

Inoculate by streaking directly from the specimen to be examined (urine). Refer to current recommendations for storage of biological specimens (2).

Incubation:

Incubate for 24 hours at 37°C.

Reading:

Lactose (+): **yellow** colonies.

Lactose (-): **blue-green** colonies.

The expected appearance of the colonies is as follows:

E. coli: **yellow**, opaque colonies, with a slightly darker centre.

Klebsiella: **yellow**, very mucoid colonies.

Proteus: **blue**, translucent colonies, generally smaller than those of *E. coli*.

Pseudomonas aeruginosa: **green** colonies, with a matt surface and irregular contours.

Enterococcus faecalis: **yellow** colonies (0.5 mm).

Staphylococcus aureus: **dark yellow** colonies.

Coagulase-negative *Staphylococci*: **pale yellow**, almost white colonies.

Lactobacillus: very small **gray** colonies with a rough surface.

7- PERFORMANCE/QUALITY CONTROL OF THE TEST

- Appearance of the medium: clear **green** agar.
- Appearance of the dehydrated medium: **beige** powder.
- The growth performances of C.L.E.D. medium are verified with the following strains:

STRAINS	CULTURE RESULT AFTER 24 hours at 37°C
<i>Escherichia coli</i> ATCC 25922	Yellow colonies
<i>Staphylococcus aureus</i> ATCC 25923	Yellow colonies
<i>Staphylococcus epidermidis</i> ATCC 12228	Yellow colonies
<i>Enterococcus faecalis var. zymogenes</i> ATCC 29212	Yellow colonies
<i>Shigella sonnei</i> ATCC 25931	Blue colonies
<i>Pseudomonas aeruginosa</i> ATCC 27853	Blue - Green colonies

8- QUALITY CONTROL OF THE MANUFACTURER

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to the final commercialization of the product. Each lot is submitted to quality control assessments and is only released to the market, after conforming to pre-defined acceptance criteria. The records relating to production and control of each single lot are kept within Bio-Rad.

9- LIMITS OF USE

- Complementary tests must be performed to identify the species of the strain isolated.
- C.L.E.D. medium is a non-selective medium. However, the growth of *Shigella* may be inhibited due to the low electrolyte content.

10- REFERENCES

1. SANDYS G.H. 1960. A new method of preventing swarming of *Proteus* spp. with a description of a new medium suitable for use in routine laboratory practice. J. Med. Lab. Technol. 17 : 224.
2. MACKEY, J.P., SANDYS, G.H. 1965. Diagnosis of of urinary tract. Br. Med J. 1 : 1173.
3. Basic Laboratory Procedures in Clinical Bacteriology. World Health Organization. Geneva.1991. 1st edition.

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