

CLED AGAR (7122)

Intended Use

CLED Agar is used for the differentiation and enumeration of microorganisms in urine.

Product Summary and Explanation

CLED Agar is an abbreviation for Cystine Lactose-Electrolyte-Deficient Agar. Sandys developed an electrolyte-deficient medium that prevented *Proteus* spp. from swarming.¹ Mackey and Sandys modified the formula by substituting lactose and sucrose for mannitol, and increasing the amount of indicator and agar.² While investigating this medium for a dip slide technique, the formula was again modified omitting sucrose and adding cystine.³

CLED Agar is recommended in the spread plate technique or a dip slide for detection of bacteria in urine. This medium supports the growth of urinary pathogens and provides distinct colony morphology. CLED Agar lacks an electrolyte (salt), necessary for growth and other characteristics of certain bacteria.⁴ CLED Agar is popular in European laboratories.⁵

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Gelatin, and Beef Extract provide the nitrogen, vitamins, and carbon in CLED Agar. Lactose is the carbohydrate. L-Cystine is added as a growth supplement for cystine-dependent coliforms. Organisms capable of fermenting lactose will lower the pH and change color of the medium from green to yellow. Bromthymol Blue is the pH indicator. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin.....	4 g
Enzymatic Digest of Casein.....	4 g
Beef Extract	3 g
Lactose	10 g
L-Cystine.....	0.128 g
Bromthymol Blue	0.02 g
Agar	15 g

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Suspend 36 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and light grey-green.

Expected Cultural Response: Cultural response on CLED Agar at the appropriate atmosphere and temperature and examined for growth at 18 – 24 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
<i>Escherichia coli</i> ATCC® 25922	10 - 300	Fair to excellent	Yellow colonies
<i>Proteus mirabilis</i> ATCC® 12453	10 - 300	Fair to excellent	Blue to blue- green colonies; suppressed swarming
<i>Staphylococcus aureus</i> ATCC® 25923	10 - 300	Fair to excellent	Yellow colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on collection and processing of urine cultures refer to appropriate references.⁵⁻⁷

Results

Refer to appropriate references for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

CLED Agar	Code No.	7122A	500 g
		7122B	2 kg
		7122C	10 kg

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., and G. H Sandys.** 1965. Laboratory diagnosis of infections of the urinary tract in general practice by means of a dip-inoculum transport medium. Br. Med. J. 2:1286.
3. **Mackey, J. P., and G. H. Sandys.** 1966. Diagnosis of urinary tract infections. Br. Med. J. 1:1173.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, Williams & Wilkins, Baltimore, MD.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
7. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.