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CLED AGAR (Cysteine Lactose Electrolyte Deficient)

Medical laboratories media for the isolation, enumeration, and identification of urinary pathogens on the basis of lactose fermentation, while controlling the swarming of Proteus spp.

REF: BS.1/CE01.100.0100	100 Gram	REF: BS.1/CE01.250.0250	250 Gram
REF: BS.1/CE01.500.0500	500 Gram		

CLINICAL SIGNIFICANCE [1]

CLED Agar is a non-selective differential plating medium for the growth and enumeration of urinary tract microorganisms. Omitting sodium chloride inhibits the Proteus swarming and supports the growth of the vast majority of bacteria causing urinary tract infections, and is used to differentiate and identify them. The presence of bacterial contaminants like Diphtheroids, Lactobacilli and other microbes indicate the degree of care taken with the handling of the urine specimen.

METHOD PRINCIPLE [2-3]

The microorganisms which cause infection in the urinary tract are generally abundant and of only one species. E. colt is the organism most frequently isolated. The seeding of the sample can be done by the dilution method or by streaking on the surface of agar with a calibrated loop. Count the colonies after 24-48 hours of incubation at a temperature of 35 ± 2°C. Report the number of colonies per ml of urine. A count of 100.000 (105)/m1 or more is an indication of a significant clinical urinary tract infection.

MEDIA COMPOSITION

Extract and peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. L-Cysteine is added as a growth supplement for cysteine dependent coliforms. Differentiation of lactose fermenters and lactose non fermenters is achieved using Bromothymol blue as a pH indicator. Organisms that ferment lactose will lower the pH and change the color of the medium from green to yellow. Bacteriological agar is the solidifying agent.

Item	Concentration %
Lactose	27.66
-Casein Peptone	11.06
-Gelatin Peptone	11.06
-Beef Extract	8.29
-L-Cystine	0.35
-Bromothymol Blue	0.05
-Bacteriological Agar	41.49

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the CLED agar material safety data sheet.

MEDIA PREPARATION, STORAGE AND STABILITY [2]

BioScien CLED Agar dehydrated media are stable until expiration date stated on label when properly stored 10-30°C. Hydrated CLED Agar media is prepared by suspend 36.15 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C, mix well and dispense into plates. When the medium is solidified, invert the plates to avoid excess moisture. The prepared medium should be stored at 8-15°C.

Formula in g/L

-Lactose : 10.00 g/L
-Casein Peptone : 4.00 g/L
-Gelatin Peptone : 4.00 g/L
-Beef Extract : 3.00 g/L
-L-Cystine : 0.128 g/L
-Bromothymol Blue : 0.02 g/L
-Bacteriological Agar:15.00 g/L

Final pH 7.3 ± 0.2 at 25°C

Deterioration

The color of ***BioScien*** CLED Agar medium is green, dehydrated medium should be homogeneous powder, free-flowing and greenish beige in color. If there are any physical changes, discard the medium.
The hydrated medium is Blue/green colored gel, media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), and contaminations.

SPECIMEN COLLECTION AND PRESERVATION [1-3]

Urine

Urine samples to be collected in sterile container, specimens is stable in urine for 2 days at 15-25°C and 6 days at 2-8°C (freeze for longer storage).
Note: for patient preparation follow Medical laboratory instruction

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile cups
- Sterile petri-dishes
- Incubator

CHARACTERISTICS OF THE COLONIES [1]

Growth Characteristics on CLED Medium (24 hours incubation)

Escherichia coli	Opaque yellow colonies with a slightly deeper yellow center
Klebsiella	Yellow to whitish-blue colonies, extremely mucoid [7]
Proteus	Translucent blue colonies
Pseudomonas aeruginosa	Green colonies with typical matted surface and rough periphery
Enterococci	Small yellow colonies, about 0.5mm in diameter
Staphylococcus aureus	Deep yellow colonies, uniform in color
Staphylococci coagulase-negative	Pale yellow colonies, more opaque than *Enterococcus faecalis*

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact ***BioScien*** technical support.

Positive controls:	**Expected results**
Proteus vulgaris ATCC® 29905 *	Good growth; blue green translucent colonies
Escherichia coli ATCC ® 25922	*Growth; yellow colonies, opaque, center slighlty deeper yellow*
Negative controls:	**Expected results**
Uninoculated medium	No change

PERFORMANCE CHARACTERISTICS [4]

Performance of the medium after incubation at a temperature of 35 ± 2°C and observed after 24-48 hours

Test Organisms	Results
Enterococcus faecalis ATCC ® 29212	Growth; small yellow colonies
Escherichia coli ATCC ® 25922	Growth; yellow colonies, opaque, center slightly deeper yellow
Proteus mirabilis ATCC ® 12453	Growth; translucent blue colonies
Staphylococcus aureus ATCC ® 25923	Growth; deep yellow colonies
Pseudomonas aeruginosa ATCC ® 27853	Growth; green colonies with matte surface and rough periphery
Klebsiella pneumoniae ATCC ® 13883	Growth; yellow to whitish-blue colonies, mucoid

REFERENCES

1. Mackey J. P. and Sandys G. H. (1966) B.M.J. 1. 1173.
2. Sandys G. H. (1960) J. Med. Lab. Techn. 17. 224.
3. Mackey J. P. and Sandys G. H. (1965) B.M.J. 2. 1286-1288.
4. Barry, A.L., P.B. Smith, and M. Turck. 1975. Cumitech 2, Laboratory diagnosis of urinary tract infections. Coordinating ed., T.L. Gavan. American Society for Microbiology, Washington, D.C

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

EC REP

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