

Cled agar with Bromothymol Blue

REF.	Pack size
612 01 100	100 gm
612 01 500	500 gm

Intended Use

CLED Agar with Bromothymol Blue is a differential culture medium intended for use in isolation, enumeration and identification of bacteria in urine.

Background

CLED Agar is an abbreviation for Cystine Lactose-Electrolyte-Deficient Agar. Sandys developed an electrolyte-deficient medium that prevented *Proteus* sp. from swarming. This medium was modified by substituting lactose and sucrose for mannitol, and increasing the amount of indicator and agar. CLED agar formula was further modified by the incorporation of cystine and omitting of sucrose; which made it ideal for dip inoculation techniques. This medium supports the growth of urinary pathogens and provides distinct colony morphology.

Principle

The essential growth nutrients are supplied by peptone, tryptone and beef extract. Lactose is the carbohydrate source. L-cystine permits the growth of "dwarf colony" coliforms. Bromothymol blue is used as the pH indicator to differentiate lactose fermenters from non-lactose fermenters. Organisms that ferment lactose will lower the pH and change the colour of the medium from green to yellow. Electrolyte sources are reduced in order to restrict the swarming of *Proteus* species.

Components	gml/Liter
Beef Extract	3.0
Pancreatic digest of Casein	4.0
Pancreatic digest of Gelatin	4.0
Bromothymol Blue	0.02
L-Cysteine	0.128
Agar	15.0
Lactose	10.0

Final pH (at 25°C) 7.3 ± 0.2

Preparation, Storage and Stability

Store the dehydrated medium at 10-30°C and use before the expiry date on the label. Store the prepared medium at 2-8°C.

After the desired amount of medium is taken out, replace the cap tightly to protect from hydration.

Procedure

1. Suspend 36.15 g of the powder in 1 L distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C for 15 minutes.

Quality Control Appearance

1- Dehydrated Appearance : Yellow coloured, homogeneous, free flowing powder.

2- Prepared Appearance : Green coloured, slightly opalescent gel.

3- Cultural Response : Cultural characteristics after 18-24 hours at 30-35°C (As per pharmacopeia or 35± 2°C for clinical specimens)

Organisms (ATCC)	Growth	Colour of the Colony
<i>Escherichia coli</i>	Good	Yellow opaque colonies
<i>Staphylococcus aureus</i>	Good	Yellow
<i>Enterococci</i>	Good	Yellow or Green
<i>Proteus vulgaris</i>	Good	Blue
<i>Klebsiella pneumoniae</i>	Good	Yellow to whitish blue

Interpretation of the results

1. Count the number of colonies on the dipstick. Multiply by dilution factor to convert the count to CFU per ml of the sample.
2. Contaminant bacteria usually appear in low numbers, which vary in colony morphology

Precautions

1. Factors that may cause urine counts from infected patients to be low include: rapid rate of urine flow, prior initiation of antimicrobial therapy, a urine pH of less than 5 and a specific gravity of less than 1.003.

2. *Shigella* species may not grow on this medium.

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

Bibliography

1. Sandys, 1960, J. Med. Lab. Tech.
2. Mackey and Sandys. 1965. Br. Med. J.
3. Mackey and Sandys. 1966. Br. Med. J.
4. 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.
5. Isenberg, H. D. (ed.). 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
6. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover

