

C.L.E.D. Agar w/Bromo Thymol Blue Plate**(M100)**

Cystine, lactose, electrolyte deficient medium, recommended for the isolation and identification of urinary pathogenic bacteria.

Composition

Composition (g/l):

Peptone.....	4.000
Trypsic peptone.....	4.000
Meat extract.....	3.000
L-Cystine.....	0.128
Lactose.....	10.000
Bromothymol blue.....	0.030
Agar.....	15.000

Description:

This general purpose medium has been recommended for bacteriological urine analysis. Current formulation is a modification of the original one reported by Sandys, that achieves an excellent colony differentiation without inhibitors. This fact, and also the careful selection of nutritive components, makes this medium a substrate able to support growth of most urinary pathogenic bacteria.

Presence of lactose as a fermentable sugar allows classic differentiation and, at the same time, lack of electrolytes suppresses swarming waves on the members of the *Proteus* species and sometimes growth of *Shigella* spp. also.

Technique:

Collect urine samples and volumes as required according to specifications, directives, official standard regulations. Spread the plates by streaking methodology or by spiral method. Incubate the plates right side up aerobically at 36±1C for 18-24h

This medium can be inoculated streaked directly with a calibrated loop to give quantitative results. After incubation, enumerate all the colonies that have appeared onto the surface of the agar. Bromo thymol blue is the pH indicator which turns yellow at acidic pH.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. For professional use only. Follow good microbiological lab practices while handling specimens and culture.

Limitations

1. Initiation of antibiotic therapy, before collection of sample, low urine pH (less than 5) etc. may result in low bacterial count from infected patients.
2. Shigella species may not grow on this medium.
3. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
4. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any organisms based on the user's requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality control**Physical/Chemical control**

Color : Green pH: 7.10-7.50

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Microbiological activity

Organisms	Inoculum CFU/ml	Growth	Colour of Colony
Escherichia coli ATCC 25922	50-100	Good growth	Large yellow colonies
Enterococcus faecalis ATCC 29212	50-100	Good growth	Small, Pinpointed, Slight yellowish or greenish
Staphylococcus aureus ATCC 25923	50-100	Good growth	Small to medium, Deep yellow colonies
Proteus vulgaris ATCC 13315	50-100	Good growth	Blue-green colonies
Klebsiella pneumoniae 13883	50-100	Good growth	Yellow to whitish blue colonies

Storage and Shelf Life

Store between 2-8°C. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Reference

1. Sandys, 1960, J. Med. Lab. Technol., 17:224.
2. Mackey and Sandys, 1965, Br. Med. J., 2:1286.
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4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.