# BD CLED Agar / MacConkey II Agar (Biplate)

## INTENDED USE

**BD CLED Agar / MacConkey II Agar (Biplate)** is used for microbiological urine analysis. CLED Agar is a differential culture medium for use in isolating and enumerating bacteria in urine. MacConkey II Agar is a selective and differential medium for the isolation and differentiation of *Enterobacteriaceae* and a variety of other gram-negative rods from clinical and non-clinical specimens.

## PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

CLED Agar: In 1960, Sandys reported on the development of a new method of preventing the swarming of *Proteus* on solid media by restricting the electrolytes in the culture medium which was modified later several times for use in urine culture.[1-3] It was designated as Cystine-Lactose-Electrolyte-Deficient (CLED) medium and reported to be ideal for urinary bacteriology. In CLED Agar, peptones and beef extract provide nutrients. Lactose is an energy source for organisms capable of utilizing it by a fermentative mechanism. Bromthymol blue is a pH indicator to differentiate lactose fermenters from lactose-nonfermenters. Organisms which ferment lactose will lower the pH and change the color of the medium from green to yellow. The cystine permits the growth of "dwarf colony" coliforms.[3] Electrolyte sources are reduced in order to minimize the swarming of *Proteus* species.

MacConkey II Agar: One of the earliest media formulations for the differentiation of *Enterobacteriaceae* was developed by MacConkey and published in 1900 and 1905.[4,5] This formulation was devised in the knowledge that bile salts are precipitated by acids and certain enteric micro-organisms ferment lactose whereas others do not possess this ability. Later on, this medium was modified several times.[6,7]

MacConkey Agar is only slightly selective since the concentration of bile salts, which inhibits gram-positive micro-organisms, is low in comparison with other enteric plating media. This medium is recommended for use with clinical specimens likely to contain mixed microbial flora, such as urine and many others, because it allows a preliminary grouping of *Enterobacteriaceae* and other gram-negative rods in lactose fermenters and lactose nonfermenters.[7-10]

The MacConkey II Agar formulation was designed to improve the inhibition of swarming *Proteus* species, to achieve better differentiation of lactose fermenters and nonfermenters, and superior growth.

In MacConkey II Agar, peptones provide nutrients. Crystal violet inhibits gram-positive bacteria, especially enterococci and staphylococci. Differentiation of enteric micro-organisms is achieved by the combination of lactose and the neutral red pH indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate.

The presence of CLED and MacConkey II Agar in this biplate allows the determination of the total count and the isolation of gram-positive and gram-negative bacteria from urine specimens.

REAGENTS

**CLED Agar / MacConkey II Agar (Biplate)**

Formula* Per Liter Purified Water

| **CLED Agar** | | **MacConkey II Agar** | |
|---|---|---|---|
| Pancreatic Digest of Gelatin | 4.0 g | Pancreatic Digest of Gelatin | 17.0 g |
| Pancreatic Digest of Casein | 4.0 | Pancreatic Digest of Casein | 1.5 |
| Beef Extract | 3.0 | Peptic Digest of Animal Tissue | 1.5 |
| Lactose | 10.0 | Lactose | 10.0 |
| L-Cystine | 0.128 | Bile Salts | 1.5 |

| | | | |
|---|---|---|---|
| Bromthymol Blue | 0.02 | Sodium Chloride | 5.0 |
| Agar | 15.0 | Neutral Red | 0.03 |
| pH 7.3 ± 0.2 | | Crystal Violet | 0.001 |
| | | Agar | 13.5 |
| | | pH 7,1 ± 0,2 | |

*Adjusted and/or supplemented as required to meet performance criteria.

## PRECAUTIONS

For In Vitro Diagnostic Use IVD. For professional use only.
Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

## STORAGE AND SHELFLIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.
Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

## USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate plates at 35 ± 2°C in an aerobic atmosphere. Examine plates at 18 to 24 h for amount of growth, pigmentation, colony size and inhibition of *Proteus* swarming/spreading.

| Strains | CLED Agar | MacConkey II Agar |
|---|---|---|
| *Escherichia coli* ATCC 25922 | Growth; colonies yellow, medium yellow | Growth; pink colonies |
| *Proteus vulgaris* ATCC 8427 | Growth; colonies colorless to blue; swarming inhibited; slight spreading acceptable | Growth; colorless to beige colonies, swarming inhibited |
| *Enterococcus faecalis* ATCC 29212 | Growth; colonies colorless to yellow; medium yellow | Inhibition partial to complete |
| *Staphylococcus aureus* ATCC 25923 | Growth; colonies small, yellow; medium yellow | Inhibition partial to complete |
| *Staphylococcus saprophyticus* NCTC 10516 | Growth; colonies small, white to yellowish; medium yellow | Inhibition partial to complete |
| Uninoculated | Green to blue-green | Light pink, slightly opalescent |

## PROCEDURE

### Materials Provided

**BD CLED Agar / MacConkey II Agar** (90 mm Biplates). Microbiologically controlled.

### Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

### Specimen Types and Collection of Specimens

This medium is exclusively used for enumerating and differentiating bacteria in urine. Midstream or catheter urine, or urine collected by suprapubic bladder puncture can be used (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). Observe aseptic techniques for collecting urine specimens. Urine must be directly streaked onto the medium not later than 2 hours after collection or must be kept refrigerated (not longer than 24 hours) to avoid overgrowth of the infectious agents or contaminants before inoculation of this medium. [8-11]

**Test Procedure**
Collect a sample of the undiluted, well-mixed urine using a calibrated loop (0.01 or 0.001 ml) for each of the two media of this biplate. Ensure proper loading of the loop with the specimen. First, streak a sample of the urine on CLED Agar, then the second sample on MacConkey II Agar. Incubate plates aerobically at 35 ± 2°C for 24 to 48 h. Do not incubate in a $CO_2$-enriched aerobic atmosphere!

**Calculation and Interpretation of Results**
Count the number of colonies (cfu) on the plate. If a 0.01 ml loop was used, each resultant colony is representative of 100 CFU/ml; if a 0.001 ml loop was used, each colony corresponds to 1000 CFU/ml of urine.[8]
Midstream and catheter urine: Current guidelines indicate that for a single isolate a density of $\geq 10^5$ cfu/ml indicates infection, $<10^5$ cfu/ml indicates urethral or vaginal contamination, and a density between $10^4$ to $10^5$ CFU/ml needs to be re-evaluated based on clinical information.[8-11]
Urine collected by suprapubic bladder puncture: Since the bladder is sterile in non-infected individuals, any cfu detected indicates an infection.
Urinary pathogens will usually yield high counts having uniform colonial morphology while contaminant bacteria usually appear in low numbers which vary in colonial morphology.
While CLED Agar allows growth of gram-positive and gram-negative bacteria, gram-positive bacteria are partially to completely inhibited on MacConkey II Agar.
Growth on MacConkey II Agar Medium indicates the presence of gram-negative rods, e.g., *Enterobacteriaceae* (like *E. coli* and many others).
CLED und MacConkey II Agar only allow a presumptive differentiation of colonies according to lactose fermentation. For a complete identification and for determination of the antimicrobial susceptibility, additional tests must be performed.[10-12]

Typical colonial morphology on BD **CLED Agar / MacConkey II Agar (Biplate)**is as follows:

| **Organisms** | **CLED Agar** | **MacConkey II Agar** |
|---|---|---|
| *Escherichia coli* | Yellow colonies, opaque; yellow medium | Growth; pink colonies |
| *Klebsiella, Enterobacter* | Yellow to whitish-blue colonies, often mucoid; yellowish medium | Mucoid, pink colonies |
| *Proteus* | Translucent blue colonies; blue-green to blue medium | Growth; colorless to beige colonies, swarming inhibited |
| *Pseudomonas aeruginosa* | Green colonies with typical matted surface and rough periphery; blue medium | Irregular, colorless to pink colonies |
| Enterococci | Small yellow colonies, yellow medium | Inhibition partial to complete |
| *Staphylococcus aureus* | Deep yellow colonies, uniform in color; yellow medium | Inhibition partial to complete |
| Coagulase negative staphylococci | Pale yellow colonies, more opaque than enterococci | Inhibition partial to complete |

## PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE
CLED Agar is suitable for the isolation and counting of many aerobically growing microorganisms, such as *Enterobacteriaceae*, *Pseudomonas* and other non-fermenting gram-negative rods, enterococci, staphylococci, *Candida* species, and many others from urine specimens.
Streptococci and other organisms requiring blood or serum for growth may only be insufficiently recovered on this medium or may need extended incubation. Therefore, the specimen should also be cultivated onto a blood agar plate if such organisms are expected.
Genitourinary pathogens such as *Neisseria gonorrhoeae, Gardnerella vaginalis, Chlamydia, Ureaplasma,* or other fastidious organisms do not grow on this medium. Consult the references for the appropriate detection techniques of these organisms.[9,10,12]

Although a differentiation according to lactose fermentation and certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures is necessary for complete identification.[10-12]

MacConkey II Agar is one of the standard media used for primary plating of clinical specimens and for a variety of nonclinical materials. On this medium, all organisms of the family *Enterobacteriaceae* and a variety of other gram-negative rods, e.g., *Pseudomonas* and related genera, will grow. Nonfermenters grow on this medium if they are resistant to its selective ingredients. Consult the respective chapters in the references before using the medium for specific organisms. [8,10]

It has been reported that some *Enterobacteriaceae* and *Pseudomonas aeruginosa* are inhibited on MacConkey Agar when incubated in a $CO_2$-enriched atmosphere.[13]

Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures is necessary for complete identification. Consult appropriate references. [8,10,12]

## REFERENCES


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## PACKAGING/AVAILABILITY

**CLED Agar / MacConkey II Agar (Biplate)**

| | |
|---|---|
| Cat. No. 257562 | Ready-to-use plated media, 20 plates |
| Cat. No. 257680 | Ready-to-use plated media, 120 plates |

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