

CLED/ Mac Agar | Ready-to-use Media

REF SP90C1011



Intended Use:

CLED Agar and MacConkey III Agar (Biplate) medium are used for urinary microbiology analysis. CLED Agar is a medium for isolating, enumerating, and presumptively identifying microorganisms from urine. MacConkey III agar is a selective and differential medium used to detect and isolate gram-negative organisms

Principle Of The Procedure:

CLED Agar:

The nutrients in CLED Agar are supplied by peptones, pancreatic digests of gelatin and casein, and beef extract. Lactose is included to provide an energy source for organisms capable of utilizing it by a fermentative mechanism. The cystine permits the growth of dwarf colony coliforms. Bromothymol blue is used as a pH indicator to differentiate lactose fermenters from lactose-no fermenters. Organisms that ferment lactose will lower the pH and change the color of the medium from green to yellow. Electrolyte sources are reduced to restrict the swarming of *Proteus* species.

MacConkey Agar:

Peptones are sources of nitrogen and other nutrients. Yeast extract is a source of trace elements, vitamins, amino acids and carbon. Lactose is a fermentable carbohydrate. When lactose is fermented, a local pH drop around the colony causes a color change in the pH indicator (neutral red) and bile precipitation. Bile salts, bile salts no. 3, oxgall and crystal violet are selective agents that inhibit growth of gram-positive organisms. Sodium chloride maintains osmotic balance in the medium. Magnesium sulfate is a source of divalent cations. Agar is the solidifying agent.

Product Summary:

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. Previous chemical methods used to inhibit swarming by *Proteus* included the addition of chloral hydrate, alcohol, sodium azide, surface-active agents, boric acid, and sulfonamides to the culture medium. This electrolyte-deficient medium of Sandys was modified by Mackey and Sandys for use in urine culture by substituting lactose and sucrose for the mannitol and increasing the concentrations of the bromothymol blue indicator and of the agar. These two investigators further modified the medium by the incorporation of cystine to enhance the growth of cystine-dependent “dwarf colony” coliforms and by deletion of sucrose. They designated the new medium as Cystine-Lactose-Electrolyte-Deficient (CLED) medium and reported it to be ideal for dip-inoculum techniques and for urinary bacteriology in general ¹⁻³

MacConkey III Agar:

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey. The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved the growth of *Shigella* and *Salmonella* strains. These modifications included the addition of 0.5% sodium chloride, decreased agar content, and altered bile salts and neutral red concentrations. The formula improvements gave improved differential reactions between these enteric pathogens and the coliform group. MacConkey Agar contains crystal violet and bile salts that inhibit gram-positive organisms and allow gram-negative organisms to grow. Isolated colonies of coliform bacteria are brick red in color and may be surrounded by a zone of precipitated bile. This bile precipitate is due to a local pH drop around the colony due to lactose fermentation. Colonies that do not ferment lactose (such as typhoid, paratyphoid and dysentery bacilli) remain colorless. When lactose nonfermenters grow in proximity to coliform colonies, the surrounding medium appears as cleared areas. MacConkey Agar is listed as one of the recommended media for the isolation of *E. coli* from nonsterile pharmaceutical products. MacConkey Agar Base is prepared without added carbohydrates, which permits their addition either individually or in combination. It is recommended that carbohydrates such as sucrose or lactose be added in a concentration of 1% to the basal medium.

Formulation* (PER LITER):

CLED Agar:

Pancreatic Digest of Gelatin	4.0g
L-Cystine	128.0mg
Pancreatic Digest of Casein	4.0g
Bromothymol Blue	0.02g
Beef Extract	3.0g
Lactose	10.0g
Agar	15.0g
pH 7.3 +/- 0.2	

MacConkey Agar:

Pancreatic Digest of Gelatin	17.0g
Peptones (meat and casein)	3.0g
Lactose	10.0g
Bile salts	1.5g
Sodium chloride	5.0g
Neutral Red	0.03g
Crystal Violet	1.0mg
Agar	13.5g
pH 7.1 +/- 0.2	

Procedure

Materials Provided

90mm CLED/Mac Agar.

Materials Required But Not Provided

Ancillary culture media, reagents, and laboratory equipment as required.

Test Procedure

1. 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.
2. First, streak a sample of the urine on CLED Agar, then the second sample on MacConkey Agar.
3. Incubate plates at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 18 to 24 hours.
4. Observe the result according to user requirements.
5. Dispose of all used reagents and contaminated materials as infectious waste. Laboratories must handle and dispose of all waste safely according to regulations.

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 urine⁴

Quality Control

Inoculate representative samples with the following strains. Incubate the inoculated plates at $35 \pm 2^{\circ}\text{C}$ for 18 to 24 hrs. to allow colonies to develop on the medium.

CLED Agar:

Strains	ATCC®	Growth
<i>Proteus mirabilis</i>	12453	Growth; colonies blue, medium blue-green to blue
<i>Escherichia coli</i>	25922	Growth; colonies yellow, medium yellow
<i>Staphylococcus aureus</i>	25923	Growth; colonies small, yellow; medium yellow
<i>Proteus vulgaris</i>	8427	Growth; colonies colorless to blue; swarming inhibited; slight spreading acceptable
Uninoculated plate	-	No growth

MacConkey III Agar:

Strains	ATCC®	Growth
<i>Escherichia coli</i>	25922	Pink to red growth
<i>Proteus mirabilis</i>	12453	Growth Colorless Inhibition of swarming
<i>Salmonella choleraesuis subsp. choleraesuis serotype Typhimurium</i>	14028	Growth Colourless
<i>Staphylococcus aureus</i>	25923	No growth
<i>Enterococcus faecalis</i>	29212	No growth
Uninoculated plate	-	No growth

Transportation:

Temperature fluctuations may occur during transportation. However, these fluctuations do not affect the performance, quality, or safety of the media.

Storage And Shelf Life:

Upon receipt, store plates at 2 to 8°C, in their original sleeve wrapping until just before 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.

Warning And Precautions:

For in vitro diagnostic use. For Professional Use Only. Do Not Reuse.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking, or other signs of deterioration.

Limitation Of The Procedure

This medium is for laboratory use only and is not intended for the diagnosis of disease or other conditions. Identifications are presumptive and colonies should be identified using appropriate methods ⁵⁻⁸

Reference

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2. Sandys, G.H. 1960. A new method of preventing swarming of *Proteus* sp. with a description of a new medium suitable for use in routine laboratory practice. *J. Med. Lab. Technol.* 17:224–233.
3. Mackey, J.P., and G.H. Sandys. 1966. Diagnosis of urinary infections. *Br. Med. J.* 1:1173.
4. Thomson, R.B., and J.M. Miller. 2003. Specimen collection, transport, and processing: bacteriology. In: Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Tenover, and R. H. Tenover (ed.). *Manual of clinical microbiology*, 8th ed. American Society for Microbiology, Washington, D.C.
5. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. *Bergey's Manual™ of determinative bacteriology*, 9th ed. Williams & Wilkins, Baltimore.
6. MacFaddin, J.F. 2000. *Biochemical tests for identification of medical bacteria*, 3rd ed. Lippincott Williams & Wilkins, Baltimore.
7. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1997. *Color atlas and textbook of diagnostic microbiology*, 5th ed. Lippincott-Raven, Philadelphia.
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Packaging Symbol

Symbol	Definition
	Catalogue number
	In Vitro Diagnostic Medical Device
	Batch code
	Date of manufacture
	Temperature limit
	Use-by date
	Keep away from sunlight
	Do not re-use
	Fragile, handle with care
	Consult instructions for use or consult electronic instructions for use
	Do not use if packaging damaged and consult instructions for use
	Manufacturer

Further Information:

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