

CLED/ Mac III Agar | Ready-to-use Media

REF SP90C1012



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:

Peptones and Lab Lemco powder are present to supply the materials required for the growth of bacteria and agar in the solidifying agent. Lactose provides a carbohydrate source. Bromothymol blue is a pH indicator which differentiates lactose fermenters (yellow) from non-fermenters. Cystine enhances the growth of cystine-dependent coliforms. Electrolytes are reduced in order to restrict the swarming of *Proteus spp.*

MacConkey III Agar:

Peptone supplies nutrients and agar is the solidifying agent. Bile salts are inhibitory to non-intestinal bacteria and help to prevent the swarming of *Proteus spp.* Bile salts and crystal violet inhibit the growth of Gram-positive cocci. Lactose is added as a carbon source. Differentiation of bacteria is achieved by the combination of lactose and the indicator dye neutral red, which is red at acid pH and yellow at alkaline. Bacteria which ferment lactose appear as red - pink-coloured colonies which may be surrounded by zones of precipitated bile salts. Precipitation is caused by the action of the acid produced by lactose fermentation on the bile salts. Bacteria which do not ferment lactose, such as *Salmonella*, usually appear as colourless to straw colonies. Sodium chloride maintains osmotic balance in the medium. Therefore, MacConkey III Agar can be used with clinical specimens likely to contain mixed flora such as urine and wounds as it allows the preliminary grouping of Gram-negative bacteria into lactose fermenters and non-fermenters.

Product Summary:

CLED Agar:

Urinary tract infections, often referred to as UTIs, are common infections that occur when bacteria invade the urinary tract by entering via the urethra. These infections can impact different parts of the urinary tract, but the most prevalent is cystitis (a bladder infection). The symptoms of cystitis can include frequent urination, or needing to urinate whilst having an empty bladder, a burning sensation or pain whilst urinating, blood in the urine and pressure or cramps in the lower abdominal area. The symptoms of kidney infections are very different. These can include chills, a fever, nausea and vomiting and pain in the lower back¹. There are many species of bacteria that cause UTIs, but most common are *Escherichia coli*, *Staphylococcus aureus* and *Proteus mirabilis*^{2,3}. Due to the severity of UTIs in a vast number of patients, it is very important to be able to isolate and identify common UTI-causing microorganisms from urine samples and wound swabs. Early diagnosis is vital in the prevention and reduction of urinary tract infections.

MacConkey III Agar:

Lactose fermenters are microorganisms that ferment lactose and those that are unable to ferment lactose are called non-lactose fermenters¹. *Escherichia coli* (*E. coli*) are non-spore forming bacteria that are able to grow in aerobic and anaerobic conditions¹. *Salmonella* is a bacterial pathogen that can be isolated from faeces, blood, bone marrow, bile, urine, food, animal feed and environmental materials. Ingestion of contaminated food and water can cause foodborne infections, including gastroenteritis, typhoid fever, paratyphoid fever or even death in humans. All *Salmonella* serotypes can cause disease in humans². *Acinetobacter baumannii* is a Gram-negative nosocomial pathogen that can persist on dry surfaces longer than any other Gram-negative bacteria. It can persist on moist and dry surfaces for more than 20 days contributes to its widespread in a hospital setting³. *Acinetobacter spp.* are commonly isolated from locations such as hand, groin, toe webs etc⁴. Due to the high antibiotic resistance shown. by this bacterium an early identification is often recommended. *Acinetobacter spp.* have been isolated in connection with community acquired and nosocomial pneumonias, urogenital tract, eye and soft tissue infections and are difficult to treat particularly due to their broad antibiotic resistance.

Formulation* (PER LITER):

CLED Agar:

Peptone	4.0g
'Lab-Lemco' powder	3.0g
Tryptone	4.0g
Lactose	10.0g
L-Cystine	0.128g
Bromothymol Blue	0.02g
Agar	15.0g
pH 7.3+/- 0.2	

MacConkey III Agar:

Peptone	20.0g
Lactose	10.0g
Bile salts No.3	1.5g
Sodium chloride	5.0g
Neutral red	0.03g
Crystal violet	0.001g
Agar	15.0g
pH 7.1 +/- 0.2	

Procedure

Materials Provided

90mm CLED/Mac III 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 methods⁵⁻⁸

Reference

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3. Gerner-Smidt, P. (1995). Taxonomy and epidemiology of Acinetobacter infections. Rev Med Microbiol., 6, 186-195.
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.
8. Isenberg, H.D. (ed.). 2004. Clinical microbiology procedures handbook, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.

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:

For further information please contact your Biomed MDX representative.

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