

HiCombi™ CLED - MacConkey Agar Plate

HB003

Intended Use

Recommended for selective isolation and differentiation of coliform and other enteric pathogens.

Composition**

C.L.E.D. Agar w/Bromo Thymol Blue

Ingredients	g / L
Peptone	4.000
Tryptone	4.000
HM Peptone B#	3.000
Lactose	10.000
L-Cystine	0.128
Bromothymol blue	0.020
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

#- Equivalent to Beef extract

MacConkey Agar

Ingredients	g / L
Gelatin peptone	17.000
Tryptone	1.500
Peptone	1.500
Lactose	10.000
Bile salts	1.500
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.001
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Streak the test inoculum (50-100 CFU) aseptically.

Principle And Interpretation

C.L.E.D. Agar w/Bromo Thymol Blue

On a solid medium, Sandys reported that swarming of *Proteus* species can be controlled by restricting the electrolytes (1). Formerly swarming of *Proteus* was controlled by adding alcohol, surface-active agent, sodium azide, boric acid etc. to the medium (1). Later on Sandy's medium was modified by Mackey and Sandys (2), by replacing mannitol by lactose and sucrose and elevating concentration of agar and bromothymol blue. This formulation was further modified by the same authors, called C.L.E.D. (Cystine-Lactose-Electrolyte-Deficient) by deleting the sucrose and by including Lcystine for promoting the growth of cystine dependent dwarf colony coliforms (3). This medium is recommended for use in urinary bacteriology, promoting the growth of all urinary pathogens. C.L.E.D. Medium is also recommended for dip stick procedures and as dip inoculum transport medium for urine specimens (2,3,4).

Peptone, Tryptone and HM Peptone B provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Lactose is the fermentable sugar. L-cystine supports the growth of dwarf coliform colony. Bromo thymol blue is the pH indicator which turns yellow at acidic pH.

Bacteriuria may be quantitated by inoculating the surface of an agar medium by proper dilution. Inoculate the medium immediately after urine collection. It can also be inoculated by calibrated loop or duplicate dilution pour plate methods (5,6).

MacConkey Agar

MacConkey agars are slightly selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical (7), dairy (8), food (9,10), water (11), pharmaceutical (12,13) and industrial sources (14). It is also recommended for the selection and recovery of the *Enterobacteriaceae* and related enteric gram-negative bacilli. USP recommends this medium for use in the performance of Microbial Limit Tests (13). These agar media are selective since the concentration of bile salts, which inhibit gram-positive microorganisms, is low in comparison with other enteric plating media. The medium M081, which corresponds with that recommended by APHA can be used for the direct plating of water samples for coliform bacilli, for the examination of food samples for food poisoning organisms (17) and for the isolation of *Salmonella* and *Shigella* species in cheese (18). Other than that this medium is also used for count of coli-aerogenes bacteria in cattle and sheep faeces (13), the count of coli-aerogenes and non-lactose fermenters in poultry carcasses (13), bacterial counts on irradiated canned minced chicken (16) and the recognition of coli-aerogenes bacteria during investigations on the genus *Aeromonas* (19). MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens (11,13). The original medium contains protein, bile salts, sodium chloride and two dyes. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Lactose-fermenting strains grow as red or pink colonies and may be surrounded by a zone of acid precipitated bile. The red colour is due to production of acid from lactose, absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8. Lactose non-fermenting strains, such as *Shigella* and *Salmonella* are colourless, transparent and typically do not alter appearance of the medium. Peptone, Tryptone and gelatin peptone are sources of nitrogen, carbon, long chain amino acids and other nutrients. Lactose is a fermentable carbohydrate, Sodium chloride maintains the osmotic equilibrium. Bile salts and crystal violet are selective agents that inhibit growth of gram-positive organisms. Neutral red is the pH indicator dye.

Type of specimen

Clinical samples - faeces, urine and other pathological material, Food and dairy samples, Water samples, Pharmaceutical samples.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (8,9). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (11). For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (12,13). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (21,22). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use. For professional use only. Read the label before opening the pack. Wear protective gloves/ protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations:

C.L.E.D. Agar w/Bromo Thymol Blue

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.

MacConkey Agar

1. Though the medium is recommended for selective isolation, further biochemical and serological testing must be carried out for further confirmation.
2. The surface of the medium should be dry when inoculated.

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

Appearance

Sterile CLED and MacConkey Agar in 90 mm disposable biplates with smooth surface and absence of black particles/ cracks/bubbles

Colour of CLED Agar

Green coloured medium

Colour of MacConkey Agar

Red coloured medium with purplish tinge

Quantity of medium

10ml of each medium in biplate

pH of CLED Agar

7.10- 7.50

pH of MacConkey Agar

6.90 -7.30

Sterility Check

Passes release criteria

Cultural response

Cultural characteristics observed after incubation at 35-37°C for 18-48 hours.

Organism	Growth on MacConkey Agar	Colour of colony on MacConkey	Growth on CLED Agar	Colour of colony on CLED Agar
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	luxuriant	pink to red	luxuriant	yellow
<i>Escherichia coli</i> ATCC 25922 (00013*)	luxuriant	pink to red with bile precipitate	luxuriant	yellow, opaque, center slightly deeper yellow
<i>Proteus vulgaris</i> ATCC 13315	luxuriant	colourless	luxuriant	blue
<i>Salmonella</i> Paratyphi A ATCC 9150	luxuriant	colourless	luxuriant	blue
<i>Salmonella</i> Paratyphi B ATCC 8759	luxuriant	colourless	luxuriant	blue
<i>Salmonella</i> Typhi ATCC 6539	luxuriant	colourless	luxuriant	blue
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	none - poor	-	luxuriant	Slight yellowish or greenish
<i>Shigella flexneri</i> ATCC 12022 (00126*)	fair to good	colourless-pink	luxuriant	blue
<i>Staphylococcus aureus</i> subsp.aureus ATCC 25923 (00034*)	inhibited	-	luxuriant	deep yellow
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	luxuriant	colourless	luxuriant	blue
<i>Klebsiella pneumoniae</i> ATCC 13883 (00097*)	luxuriant	pink to red	luxuriant	yellow to whitish blue
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	luxuriant	colourless	luxuriant	blue

Key :- * Corresponding WDCM numbers # Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

On receipt store between 20-30°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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (21,22).

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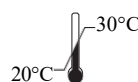
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IVD *In vitro* diagnostic
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