

## HiCombi™ Nutrient - MacConkey Agar Plate

HB001

### Intended Use

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

### Composition\*\*

#### Nutrient Agar

Ingredients	g / L
Peptone	5.000
Sodium chloride	5.000
HM peptone B <sup>#</sup>	1.500
Yeast extract	1.500
Agar	15.000
Final pH ( at 25°C)	7.4±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

#### Nutrient Agar

Nutrient media are basic culture media used for maintaining microorganisms, cultivating fastidious organisms by enriching with serum or blood and are also used for purity checking prior to biochemical or serological testing (1,2). Nutrient Agar is ideal for demonstration and teaching purposes where a more prolonged survival of cultures at ambient temperature is often required without risk of overgrowth that can occur with more nutritious substrate. This relatively simple formula has been retained and is still widely used in the microbiological examination of variety of materials and is also recommended by standard methods. It is one of the several non-selective media useful in routine cultivation of microorganisms (3,4). It can be used for the cultivation and enumeration of bacteria which are not particularly fastidious. Addition of different biological fluids such as horse or sheep blood, serum, egg yolk etc. makes it suitable for the cultivation of related fastidious organisms. Peptone, HM Peptone B and yeast extract provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients necessary for the growth of bacteria. Sodium chloride maintains the osmotic equilibrium of the medium.

#### MacConkey Agar

MacConkey agars are slightly selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical (5), dairy (6), food (7,8), water (9), pharmaceutical (10,11) and industrial sources (12). 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 (14) and for the isolation of *Salmonella* and *Shigella* species in cheese (15). 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* (17). MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens (8,10). 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,

### Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4,6). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (8). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (18,19). 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

#### General

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. 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 specific microorganism other than mentioned in the COA based on the user's requirement.
3. Further serological and biochemical testing is required for complete identification.

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

#### Colour of Nutrient Agar

Light amber coloured medium

#### Colour of MacConkey Agar

Red coloured medium with purplish tinge

#### Quantity of medium

10ml of each medium in biplate

#### pH of Nutrient Agar

7.20- 7.60

#### 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 Agar	Growth on Nutrient Agar
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	luxuriant	pink to red	good-luxuriant
<i>Escherichia coli</i> ATCC 25922 (00013*)	luxuriant	pink to red with bile precipitate	good-luxuriant
<i>Proteus vulgaris</i> ATCC 13315	luxuriant	colourless	good-luxuriant
<i>Salmonella</i> Paratyphi A ATCC 9150	luxuriant	colourless	good-luxuriant
<i>Salmonella</i> Paratyphi B ATCC 8759	luxuriant	colourless	good-luxuriant
<i>Salmonella</i> Typhi ATCC 6539	luxuriant	colourless	good-luxuriant
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	none - poor	colourless to pale pink	good-luxuriant
<i>Shigella flexneri</i> ATCC 12022 (00126*)	fair to good	colourless	good-luxuriant
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	inhibited	-	good-luxuriant
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	luxuriant	colourless	good-luxuriant
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	luxuriant	colourless	good-luxuriant
<i>Streptococcus pyogenes</i> ATCC 19615	none-poor	-	good-luxuriant

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 (18,19).

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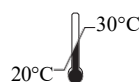
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Plot No.C-40, Road No.21Y,  
MIDC, Wagle Industrial Area,  
Thane (W) -400604, MS, India



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