HiCombi™ Blood- MacConkey Agar Plate HB006

Intended Use

Recommended for isolation and cultivation of fastidious organisms and differentiation of coliforms and other enteric pathogens.

Composition**

Blood Agar Base

Ingredients	g / L
HM peptone B#	10.000
Tryptose	10.000
Sodium chloride	5.000
Agar	15.000
Sterile defibrinated blood	50.000 ml
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef Heart peptone

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 aseptically.

Principle And Interpretation

Blood Agar Base

Blood Agar Base is a highly nutritious medium generally used as a basal medium for preparing blood agar by supplementation with blood. It can also be used as general-purpose media without the addition of blood. Blood Agar Base media can be used with added phenolphthalein phosphate (1) for the detection of phosphate producing Staphylococci, with added salt and agar for assessment of surface contamination on equipment and pig carcass (2) and to determine salinity range of marine *Flavobacteria* (3). It can also be used for preparation of *Salmonella* Typhi antigens (4). Blood Agar Base is recommended by APHA (5) and Standard Methods (6,7) for testing of food samples.

HM peptone B and tryptose provides carbon, nitrogen, amino acids and vitamins. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. Addition of blood makes the medium more nutritious by providing additional growth factors required by fastidious organisms. It also helps in visualizing the haemolytic reactions. However, haemolytic reactions depend on the animal blood used. Sheep blood gives best results for Group A Streptococci (8). But sheep blood fails to support growth of *Haemophilus haemolyticus* since sheep blood is deficient in pyridine nucleotides. However when horse blood is used *H. haemolyticus* colonies produce haemolysis and mimic *Streptococcus pyogenes* (9).

MacConkey Agar

MacConkey agars are slightly selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical (9), food (5), water (12), pharmaceutical (13,14) and industrial sources (15). It is also recommended for the selection and recovery of the *Enterobacteriaceae* and related enteric gram-negative bacilli. 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 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 (4) and for the isolation of *Salmonella* and *Shigella* species in cheese (4). Other than that this medium is also used for count.Other than that this medium is also used for count of coli-aerogenes bacteria in cattle and sheep faeces (21), the count of coli-aerogenes and non-lactose fermenters in poultry carcasses (18), bacterial counts on irradiated canned minced chicken (16) and the recognition of coli-aerogenes bacteria during investigations on the genus *Aeromonas* (21). 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

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4,6). 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 container. 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

Blood Agar Base

1.Addition of sheep blood is recommended to detect haemolysis. This medium does not support the growth of *H.haemolyticus.*

2.Haemolytic pattern varies with the source of blood used.

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

Colour

Blood Agar : Red coloured medium

MacConkey Agar : Red coloured medium with purplish tinge.

Quantity of medium

10 ml medium in each sidepH of Blood 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 blood agar	Hemolysis
Streptococcus pyogenes ATCC 19615	-	-	luxuriant	Beta
Streptococcus pneumoniae ATCC 6303	-	-	luxuriant	Alpha
# *Klebsiella aerogenes* ATCC 13048 (00175*)	luxuriant	pink-red		
Escherichia coli ATCC 25922 (00013*)	luxuriant	pink-red with bile ppt		
Proteus vulgaris ATCC 13315	luxuriant	colourless		
Salmonella Paratyphi A ATCC 9150	luxuriant	colourless		
Salmonella Paratyphi B ATCC 8759	luxuriant	colourless		
*Salmonella*Typhi ATCC 6539	luxuriant	colourless		
Salmonella Enteritidis ATCC 13076 (00030*)	luxuriant	colourless		
Enterococcus faecalis ATCC 29212 (00087*)	none-poor	colourless -pink		
Shigella flexneri ATCC 12022 (00126*)	fair-good	colourless		
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	inhibited			

Key :- * Corresponding WDCM numbers

Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

On receipt store between 2-8°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 (22,23).

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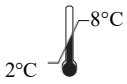

Storage temperature

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