

HiCombi™ Chocolate - MacConkey Agar Plate

HB010

Intended Use

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

Composition**

Chocolate Agar

Ingredients	g / L
Proteose peptone	20.000
Dextrose (Glucose)	0.500
Sodium chloride	5.000
Disodium hydrogen phosphate	5.000
Agar	15.000
Haemoglobin solution (FD022)	495 ml
Yeast Autolysate Supplement (FD027)/ Vitamino Growth Supplement (FD025)	1 vial
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

MacConkey Agar

Ingredients	g / L
Peptone	20.000
Lactose	10.000
Sodium taurocholate	5.000
Neutral red	0.040
Agar	20.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

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

Principle And Interpretation

Chocolate Agar Base

Neisseria gonorrhoeae is a gram-negative bacteria and the causative agent of gonorrhoea, however it is also occasionally found in the throat. The cultivation medium for gonococci should ideally be a rich nutrients base with blood, either partially lysed or completely lysed. The diagnosis and control of gonorrhoea have been greatly facilitated by improved laboratory methods for detecting, isolating and studying *N. gonorrhoeae*.

Chocolate Agar Base, with the addition of supplements, gives excellent growth of the *gonococcus* without overgrowth by contaminating organisms. G.C. Agar (M434) can also be used in place of Chocolate Agar Base, which gives slightly better results than Chocolate Agar (1). The diagnosis and control of gonorrhoea have been greatly facilitated by improved laboratory methods for detecting, isolating and studying *N. gonorrhoea*. Interest in the cultural procedure for the diagnosis of gonococcal infection was stimulated by Ruys and Jens, Mcleod and co-workers (2), Thompson (3), Leahy and Carpenter (4), Carpenter, Leahy and Wilson (5) and Carpenter (6), who clearly demonstrated the superiority of this method over the microscopic technique. Chocolate Agar Base with addition of supplement not only supports the growth of the *gonococcus* in pure culture but also permits its development from the mixed flora encountered in chronic gonococcal infections. Carpenter (7) reported that this medium and Haemoglobin (FD022) is useful for cultural detection of the *Gonococcus*.

MacConkey Agar

MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens (8,9). Subsequently MacConkey Agar and Broth have been recommended for use in microbiological examination of foodstuffs (10) and for direct plating / inoculation of water samples for coliform counts (11). These media are also accepted by the Standard Methods for the Examination of Milk and Dairy Products (12) and pharmaceutical preparations (13).

Original medium contains protein, bile salts, sodium chloride and two dyes. The selective action of this medium is attributed to bile salts, which are inhibitory to most species of gram-positive bacteria. MacConkey Agar w/o CV, NaCl and W/ 0.5% Sodium taurocholate is a modification of the original formulation with the exclusion of crystal violet and inclusion of sodium taurocholate instead of bile salts. 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 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 and transparent and typically do not alter appearance of the medium. *Yersinia enterocolitica* may appear as small, non-lactose fermenting colonies after incubation at room temperature.

Type of specimen

Clinical samples - Vaginal samples, faeces, urine, 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 (8,10).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (12). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (22,23).

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 :

Chocolate Agar Base

1. Further biochemical tests must be carried out for confirmation.

MacConkey Agar

1. The medium differentiates organisms on the basis of lactose fermentation. Further biochemical test must be carried out for confirmation.
2. The surface of the medium should be dry before inoculation.

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

Colour of Chocolate Agar

Chocolate brown coloured medium

Colour of MacConkey Agar

Red coloured medium with purplish tinge

Quantity of medium

10ml of each medium in biplate

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

Organism	Growth on Chocolate Agar
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<i>Neisseria gonorrhoeae</i> ATCC 19424	luxuriant
<i>Streptococcus pneumoniae</i> ATCC 6303	luxuriant
<i>Streptococcus pyogenes</i> ATCC 19615	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 (22,23).

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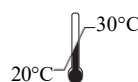
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