HiCombi™ Cetrimide - MacConkey Agar Plate HB005

Intended Use

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

Composition**

Cetrimide Agar Base

Ingredients	g / L
Gelatin peptone	20.000
Magnesium chloride	1.400
Potassium sulphate	10.000
Cetrimide	0.300
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

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
Glycerol	10.00ml
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

Cetrimide Agar Base

Pseudomonas aeruginosa grows well on all normal laboratory media but specific isolation of the organism, from environmental sites or from human, animal or plant sources, is best carried out on a medium, which contains a selective agent and also constituents to enhance pigment production. Most selective media depend upon the intrinsic resistance of the species to various antibacterial agents. Cetrimide inhibits the growth of many microorganisms whilst allowing *Pseudomonas aeruginosa* to develop typical colonies.

Cetrimide is a quaternary ammonium salt, which acts as a cationic detergent that reduces surface tension in the point of contact and has precipitant, complexing and denaturing effects on bacterial membrane proteins. It exhibits inhibitory actions on a wide variety of microorganisms including *Pseudomonas* species other than *Pseudomonas aeruginosa*. King et al developed Medium A for the enhancement of pyocyanin production by *Pseudomonas* (1). Cetrimide Agar developed by Lowburry (1) is a modification of Tech Agar (Medium A) with addition of 0.1% cetrimide for selective isolation of *P.aeruginosa*. Later, due to the availability of the highly purified cetrimide, its concentration in the medium was decreased (2,3). The incubation was carried out at 37°C for a period of 18-24 hours (4).

P.aeruginosa can be identified due to their characteristic production of pyocyanin, a blue, water-soluble, non-fluorescent phenazine pigment coupled with their colonial morphology and the characteristic grape-like odor of aminoacetophenone (5). *P.aeruginosa* is the only species of *Pseudomonas* or gram-negative rod known to excrete pyocyanin. These media are therefore, important in the identification of *P.aeruginosa*. These media are used for the examination of cosmetics (6) and clinical specimens (5,7) for the presence of *P.aeruginosa,* as well as for evaluating the efficacy of disinfectants against this organism (8). Gelatin peptone provide necessary nutrients for *P.aeruginosa*. Sodium chloride maintains osmotic equilibrium in the medium. Magnesium chloride and potassium sulfate stimulates pyocyanin production (9).

For the isolation of *P.aeruginosa*, plates of Cetrimide Agar should be inoculated from non-selective medium such as Brain Heart Infusion Broth (M210) or Soyabean Casein Digest Medium (M011). If the count is high, the test sample can be directly inoculated onto Cetrimide Agar. *P.aeruginosa* colonies may appear pigmented blue, blue-green or non-pigmented. Colonies exhibiting fluorescence at 250nm and a blue green pigmentation are considered as presumptive positive. *P.aeruginosa* may lose its fluorescence under UV if the cultures are left at room temperature for a short time. Fluorescence reappears after the plates are re-incubated (4). Type of peptone used in the base may also affect pigment production (4,10). Certain strains of *P.aeruginosa* may not produce pyocyanin. Other species of *Pseudomonas* do not produce pyocyanin but fluoresce under UV light. Most non-Pseudomonas species are inhibited on Cetrimide Agar, and some species of *Pseudomonas* may also be inhibited. Some non-fermenters and some aerobic spore formers may exhibit a water-soluble tan to brown pigmentation on this medium. *Serratia* may exhibit pink pigmentation (3). Biochemical tests and serological procedures should be performed to confirm the findings.

MacConkey Agar

MacConkey agars are slightly selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical (12), dairy (13), food (14,15), water (16), pharmaceutical (17,18) and industrial sources (19). 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 (18). 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 (20) and for the isolation of *Salmonella* and *Shigella* species in cheese (21). Other than that this medium is also used for count of coli-aerogenes bacteria in cattle and sheep faeces (18), the count of coli-aerogenes and non-lactose fermenters in poultry carcasses (18), bacterial counts on irradiated canned minced chicken (22) and the recognition of coli-aerogenes bacteria during investigations on the genus *Aeromonas* (23). MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens (16,18). 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 - urine, faeces, wound; 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 (11,13). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (15). For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (16,17). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (25,26). 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 :

Cetrimide Agar Base

1.This medium is a selective medium, some strains may show poor growth as cetrimide is highly toxic.

2.Further biochemical tests must be carried out for further confirmation.

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 Cetrimide Agar and MacConkey Agar in 90 mm disposable biplates with smooth surface and absence of black particles/cracks/bubbles
Colour of Cetrimide Agar Base
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 Cetrimide Agar
7.00- 7.40
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 Cetrimide Agar Base	Colour of colony on Cetrimide Agar Base
# *Klebsiella aerogenes* ATCC 13048 (00175*)	luxuriant	pink to red	-	-
Escherichia coli ATCC 25922 (00013*)	luxuriant	pink to red with bile precipitate	Inhibited	-
Proteus vulgaris ATCC 13315	luxuriant	colourless	-	-
Salmonella Paratyphi A ATCC 9150	luxuriant	colourless	-	-
Salmonella Paratyphi B ATCC 8759	luxuriant	colourless	-	-
Stenotrophomonas maltophilia ATCC 13637	-	-	Inhibited	-
Salmonella Typhi ATCC 6539	luxuriant	colourless	-	-
Enterococcus faecalis ATCC 29212 (00087*)	none - poor	colourless to pale pink	-	-
Shigella flexneri ATCC 12022 (00126*)	fair to good	colourless	-	-
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	inhibited	-	Inhibited	-
Salmonella Enteritidis ATCC 13076 (00030*)	luxuriant	colourless	-	-
Pseudomonas aeruginosa ATCC 27853 (00025*)	-	-	luxuriant	Yellow green to 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 (25,26).

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Revision : 03/2024

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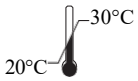
IVD ***In vitro*** **diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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CE **CE Marking**

Do not use if package is damaged

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