

MacConkey HiVeg[®] Agar w/o CV, NaCl, w/0.004% NR and 2.0% Agar

MV082

Intended Use:

Recommended for cultivation and differentiation of enteric bacteria, the omission of sodium chloride prevents the spreading of *Proteus* colonies.

Composition**

Ingredients	g / L
HiVeg [®] peptone	24.500
Lactose	10.000
Synthetic detergent	0.500
Neutral red	0.040
Agar	20.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 55.04 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. It is preferred to dry surface of media plate before inoculation. **AVOID OVERHEATING.**

Note: For the cultivation of Vibriospecies, add 5.0 grams per litre of Sodium chloride before sterilization.

Principle And Interpretation

MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens (1,2). Subsequently MacConkey Agar and Broth have been recommended for use in microbiological examination of foodstuffs (3) and for direct plating / inoculation of water samples for coliform counts (4). These media are also accepted by the Standard Methods for the Examination of Milk and Dairy Products (5) and pharmaceutical preparations (6). 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. MacConkey HiVeg[®] Agar w/o CV and NaCl, w/ 0.004% NR and 2.0% Agar is same as MacConkey Agar w/o CV, NaCl and w/ 0.5% Sodium taurocholate except that the animal based peptones are completely replaced with vegetable peptones to avoid the BSE/TSE risks associated with the animal peptones.

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

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 (3,5). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(4) After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

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

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium

Orange red coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.5% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Salmonella</i> Paratyphi B ATCC 8759	50-100	luxuriant	≥50%	colourless
<i>Salmonella</i> Typhi ATCC 6539	50-100	luxuriant	≥50%	colourless
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50-100	luxuriant	≥50%	colourless
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	50-100	fair-good	30-40%	pale pink -red
<i>Salmonella</i> Paratyphi A ATCC 9150	50-100	luxuriant	≥50%	colourless
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	≥50%	pink to red with bile precipitate
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	50-100	fair to good	30-40%	pale pink to red
<i>Shigella flexneri</i> ATCC 12022 (00126*)	50-100	fair to good	30-40%	colourless
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50-100	luxuriant	≥50%	pale pink to red
\$ <i>Proteus hauseri</i> ATCC 13315	50-100	luxuriant	≥50%	colourless

Key : *Corresponding WDCM numbers.

- Formerly known as *Enterobacter aerogenes*

\$- Formerly known as *Proteus vulgaris*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1. MacConkey, 1900, The Lancet, ii:20.
2. MacConkey, 1905, J. Hyg., 5:333.
3. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4. Lipps WC, Braun-Howland EB, Baxter TE, eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
5. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
6. The United States Pharmacopoeia-National Formulary (USP-NF), 2022
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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Disclaimer :

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