



## MacConkey Broth w/ Neutral Red

M007S

### Intended use

MacConkey Broth w/ Neutral Red is used for selective enrichment and enumeration of coliforms. It is recommended by BIS committee under the specifications IS:5887 (Part I and Part II)-1976, reaffirmed 2005.

### Composition\*\*

Ingredients	g / L
Peptone	20.000
Lactose	10.000
Bile salts	5.000
Sodium chloride	5.000
Neutral red	0.070
Final pH ( at 25°C)	7.5±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 40.07 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Distribute into test tubes with inverted Durham tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubes before inoculation.

### Principle And Interpretation

MacConkey Broth is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens (1,2). Subsequently MacConkey Broth have been recommended for use in microbiological examination of foodstuffs (3) and for direct inoculation of water samples for coliform counts (4). This medium is also accepted by the Standard Methods for the Examination of Milk and Dairy Products (5,6). MacConkey Broth w/ Neutral Red is recommended by BIS under the specification IS:5887 (Part I) 1976 for Detection of Bacteria responsible for food poisoning Part I : for Isolation, Identification and Enumeration of *Escherichia coli*. This culture media is considered as a standard medium for the primary isolation as well as presumptive identification of coli aerogenes group in food and water which contains neutral red as an indicator (7,8,9).

Bile salt inhibits 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. 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 typically do not alter appearance of the medium.

### 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 (5,6,10). 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 the samples.

### 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. 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 unique requirement.

## 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

Pale yellow to pink coloured homogeneous free flowing powder

#### Colour and Clarity of prepared medium

Red clear solution without any precipitate

#### Reaction

Reaction of 4.0% w/v aqueous solution at 25°C. pH : 7.5±0.2

#### pH

7.30-7.70

#### Cultural Response

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

Organism	Inoculum (CFU)	Growth	Acid	Gas
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50-100	luxuriant	positive reaction	positive reaction
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	positive reaction	positive reaction
<i>Salmonella Choleraesuis</i> ATCC 12011	50-100	fair to good	negative reaction	negative reaction
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	>=10 <sup>4</sup>	inhibited	-	-

Key :(\*) Corresponding WDCM numbers (#) Formerly known as *Enterobacter aerogenes*

### 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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (11,12)

### Reference

1. MacConkey, 1900, The Lancet, ii:20.
2. MacConkey, 1905, J. Hyg., 5:333.
3. Speck M. (Ed.), 1985, Compendium of Methods for the Microbiological Examination of Foods, 2nd ed., APHA, 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. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
7. Bureau of Indian Standards IS : 5401 - 1969.
8. Bureau of Indian Standards IS : 5887 (Part I) - 1976, reaffirm 1986.
9. Bureau of Indian Standards IS : 5887 (Part II) - 1976, reaffirm 1986.

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10. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
  11. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
  12. 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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