HiCrome™ M-Coliform Differential Agar Base

M1951

Intended Use:

Recommended as a selective and differential agar for the detection of coliform bacteria using membrane filtration technique from water samples.

Composition**

Ingredients	g / L
Peptone	5.000
Tryptone	10.000
Yeast extract	3.000
Lactose	12.500
Sodium deoxycholate	0.150
Aniline Blue	0.100
Chromogenic substrate	0.500
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.25 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE.** Cool to 45-50°C. Aseptically add the rehydrated contents of one vial of Mon Selective Supplement (FD309). Mix well and pour into sterile Petri plates.

Principle And Interpretation

HiCrome™ M-Coliform Differential agar is based on coliform enumeration medium, M-FC Agar (1). This medium was modified for detection and enumeration of total coliforms by addition Monensin supplement to improve the recovery of injured coliforms (2).

Peptone, tryptone and yeast extract provides carbon, nitrogen compounds, long chain amino acids, vitamins and other essential growth nutrients. Lactose is the fermentable carbohydrate. Monensin and sodium deoxycholate acts as selective agents, inhibiting Gram-positive bacteria. Aniline blue forms the indicator system of the medium. The chromogenic mixture induces *E.coli* to produce ß-glucuronidase and helps injured coliforms to grow in the presence of selective agents.

Type of specimen

Water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3). 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. ß-glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative.
2. Some species may show poor growth due to nutritional variations.

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 greyish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured, clear solution without any precipitate

Reaction

Reaction of 4.63 % w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony(On membrane filter)
##*Proteus hauseri* ATCC 13315	50-100	good-luxuriant	>=50%	tan
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=50%	blue
****Bacillus spizizenii* ATCC 6633 (00003*)	>=10^4	inhibited	0%	

Key : * Corresponding WDCM numbers , ## Formerly known as *Proteus vulgaris*, **Formerly known as *Bacillus subtilis* subsp. *spizizenii*

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°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 (4,5).

Reference

1. Entis, P., and P. Boleszczuk. 1990. Direct enumeration of coliforms and Escherichia coli using the automated hydrophobic membrane filter technique.J. Food Prod. 45:292-296. by hydrophobic grid membrane filter in 24 hours using MUG. J. Food Prot. 53:948-952.

2. Brodsky, M. H., P. Entis, A. N. Sharpe, and G. A. Jarvis. 1982. Enumeration of indicator organisms in foods

3. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

5. 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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