HiCrome™ MM Agar

M1393

Intended Use:

Recommended for identification and differentiation of *Salmonella* and non-salmonella like *Citrobacter* from water and clinical samples.

Composition**

Ingredients	g / L
Peptone	10.000
HM peptone B #	2.000
D-Cellobiose	3.000
Lactose	10.000
D-Mannitol	1.200
D-Trehalose	1.330
Chromogenic mixture	6.600
Agar	15.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef extract

Directions

Suspend 49.13 gram 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.

Principle And Interpretation

HiCrome™ MM Agar was formulated by Miller and Mallison (1) for specific isolation and detection of *Salmonellae*. This medium is superior to XLT4 Agar in supporting growth of *Salmonella* due to the presence of appropriate proportion of four sugars. Most differential and selective media are formulated with one or more sugars and pH indicators respectively. The utilization of sugars by organisms results in pH-changes. This is used as a means of distinguishing *Salmonella f*rom competing bacteria on the basis of colony colour.

Salmonella usually are unable to ferment these sugars (2) that supports growth of competing bacteria. Thus other bacteria tend to overgrow *Salmonellae,* masking their presence. The inclusion of sugars like mannitol, cellobiose and trehalose stimulate the better initial growth of *Salmonella* cells. However, the low concentrations of these sugars do not interfere with the utilization of protein and H_2S production. Presence of lactose suppresses H_2S production by non-salmonellae like *Citrobacter freundii.* The chromogenic mixture, present in this medium helps to differentiate between lactose fermenters and nonfermenters. Lactose fermenters give bluish green coloured colonies, which would have been impossible to differentiate with an indicator based on pH change. Inclusion of tergitol 4 in the medium suppresses the presence of *Proteus* and *Providencia* colonies. Peptone and HM peptone B provide essential nitrogen compounds.

Type of specimen

Clinical samples - Faeces, urine, etc.; Food samples; Water samples

Specimen Collection and Handling:

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3).For food samples, follow appropriate techniques for sample collection and processing as per guidelines (4). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). 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 :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Though most of the *Salmonella* produce H_2S certain non H_2S producing *Salmonella* species may appear as colourless colonies.
3. Certain *Salmonella* species which are lactose fermenters may show as bluish green coloured colonies.
4. Further confirmation may be carried out on suspected colonies.

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

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured, slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.91% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH

7.40-7.80

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Citrobacter freundii ATCC 8090	50-100	good-luxuriant	>=50%	colourless may show bluish green colour on prolonged incubation
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=50%	light blue
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	>=50%	black centered
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	luxuriant	>=50%	black centered
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant	>=50%	colourless

Key : (*)Corresponding WDCM numbers.

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1. Miller R.G. and Mallison E.T., 2000, J. Food Protection, 63(10), 1443-46.
2. Miller R.G., Tate C.R., Mallinson E.T. and Scherrer J.A., 1991, Pault Sa 70:2429-32.
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. 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.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. 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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IVD ***In vitro* diagnostic medical device**

15°C – 25°C **Storage temperature**

EC REP

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CE **CE Marking**

Do not use if package is damaged