



HiCrome™ MM Agar Modified

M1816

Intended use

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

Composition**

Ingredients	g / L
Proteose peptone	6.000
Yeast extract	10.000
L-Lysine hydrochloride	5.000
D-Cellobiose	10.000
Lactose	10.000
Sucrose	10.000
D-Xylose	3.750
Ferric ammonium citrate	0.800
Sodium thiosulphate	6.800
Chromogenic mixture	0.200
Bromothymol blue	0.100
Agar	18.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 80.65 gram in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely.

DO NOT AUTOCLAVE. 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. HiCrome™ MM Agar, Modified is a slight modification of HiCrome™ MM Agar and designed to differentiate *Enterobacteriaceae* especially *Salmonella* from *Proteus* and *Citrobacter* group. The utilization of sugars by organisms results in pH-changes. This is used as a means of distinguishing *Salmonella* from competing bacteria on the basis of colony colour.

Salmonella are gram negative, anaerobic, non sporulating rods in the family *Enterobacteriaceae* present in the stomach and intestinal tissues of human & animals and are found in their wastes. *Salmonella* usually are unable to ferment the sugars (2) that support growth of competing bacteria. Thus other bacteria tend to overgrow *Salmonellae*, masking their presence. Proteose peptone is a source of carbon, nitrogen and other essential amino acid and growth factor. Yeast extract which provides nitrogen and vitamin required for growth. To add to the differentiating ability of the formulation, an H₂S indicator system, consisting of sodium thiosulphate and ferric ammonium citrate, is included for the visualization of hydrogen sulphide produced, resulting in the formation of colonies with black centers. Bromothymol blue act as a pH indicator. The inclusion of sugars like lactose, sucrose, xylose and cellobiose provides source of fermentable carbohydrate which stimulate the better initial growth of *Salmonella* cells. Presence of lactose suppresses H₂S production by non salmonellae like *Citrobacter freundii*. A chromogenic mixture, present in this medium helps to differentiate between lactose fermenters and non-fermenters. Lactose fermenters give bluish green coloured colonies, which would have been impossible to differentiate with an indicator based on pH change.

Type of specimen

Clinical samples - skin lesions, inflammatory secretions, faeces, etc. ; Food samples; Water samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(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. Due to nutritional variations, some strains show poor growth
2. Though most of the *Salmonella* produce H₂S certain non H₂S producing *Salmonella* species may appear as colourless colonies.
3. Certain *Salmonella* species which are lactose fermenters may show as bluish green coloured colonies.
4. Final confirmation of suspected colonies must be carried out by serological and biochemical tests.

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 1.8% Agar gel

Colour and Clarity of prepared medium

Bluish Green coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 8.07 % 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
<i>Citrobacter freundii</i> ATCC 8090	50-100	good-luxuriant	≥50%	Yellow coloured
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	≥50%	Bluish green
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	≥50%	black centered
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50-100	luxuriant	≥50%	black centered with yellow zone
<i>Salmonella</i> Typhi ATCC 6539	50-100	good-luxuriant	≥50%	Black centered
<i>Proteus mirabilis</i> ATCC 25933	50-100	good-luxuriant	≥50%	Gray coloured
<i>Klebsiella pneumoniae</i> ATCC 13883	50-100	luxuriant	≥50%	Yellowish green

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 (3,4).

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. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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.
5. 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.
6. Lipps WC, Braun-Howland EB, Baxter TE, eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

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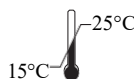
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