HiCrome™ MM Agar, Modified

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

M1816

Composition **

Ingredients	Grams/Litre
Proteose peptone	6.00
Yeast extract	10.00
L-Lysine hydrochloride	5.00
D-Cellobiose	10.00
Lactose	10.00
Sucrose	10.00
D-Xylose	3.75
Ferric ammonium citrate	0.80
Sodium thiosulphate	6.80
Chromogenic mixture	0.20
Bromothymol blue	0.10
Agar	18.00

Final pH (at 25°C) 7.6 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 80.65 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. 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 *Salmonella*e. 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 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 *Salmonella*e, masking their presence. Proteose peptone is a source of carbon, nitrogen and other essential amino acid and growth factor. Yeast extract provides vitamin especially Group B vitamins required for growth. To add to the differentiating ability of the formulation, an H_2S 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 H_2S production by non-*Salmonella*e like *Citrobacter freundii*. A 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.

Type of specimen

Clinical samples : Faeces, water and food samples

Specimen Collection and Handling

For clinical samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3, 4).

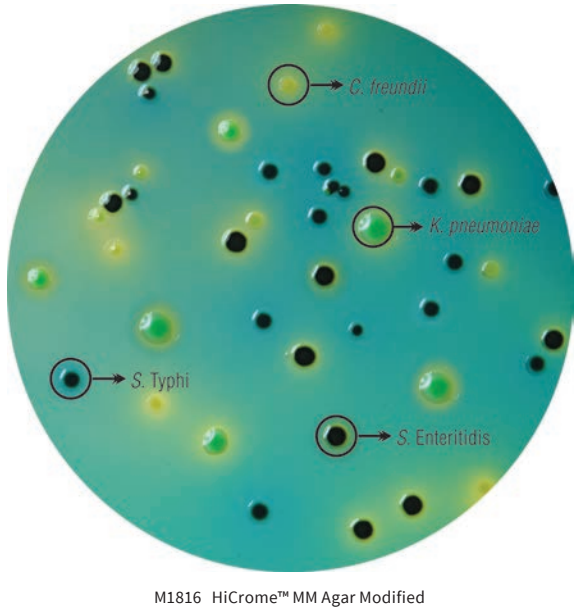
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).

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 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



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Limitations

1. Due to nutritional variations, some strains may show poor growth.
2. Though most of the *Salmonella* produce H_2S, certain non H_2S producing *Salmonella* species may sappear 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 recommended temperature.

Quality Control

Appearance of Powder : 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.
Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18 - 24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Citrobacter freundii (8090)	50-100	good - luxuriant	≥50%	Yellow# coloured
Escherichia coli (25922) (00013*)	50-100	luxuriant	≥50%	Bluish green
Salmonella Typhimurium (14028) (00031*)	50-100	luxuriant	≥50%	Black centered
Salmonella Enteritidis (13076) (00030*)	50-100	luxuriant	≥50%	Black centered with yellow zone
Salmonella Typhi (6539)	50-100	good - luxuriant	≥50%	Black centered
Proteus mirabilis (25933)	50-100	good - luxuriant	≥50%	Gray coloured
Klebsiella pneumoniae (13883) (00097*)	50-100	luxuriant	≥50%	Yellowish green, mucoid

key # : may show bluish green colour on prolonged incubation.
* = corresponding WDCM Numbers

Storage and Shelf-life

Store between 2-8°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 inorder 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3, 4).

References

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 MicrobiologyProcedures 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. Fifth (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.