

For identification and differentiation of *Salmonella* species

HiCrome™ MM Agar

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

M1393

Composition **

Ingredients	Grams/Litre
Peptone	10.00
HM peptone B#	2.00
D-Cellobiose	3.00
Lactose	10.00
D-Mannitol	1.20
D-Trehalose	1.33
Chromogenic mixture	6.60
Agar	15.00

Final pH (at 25°C) 7.6 ± 0.2

** Formula adjusted, standardized to suit performance parameters

#Equivalent to Beef extract

Directions

Suspend 49.13 grams in 1000 ml 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* from competing bacteria on the basis of colony colour. *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. 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 H₂S production. 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 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 (included in chromogenic mixture) in the medium suppresses the presence of *Proteus* and *Providencia* colonies. Peptone and HM peptone B provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients.

Type of specimen

Clinical: faeces, urine; Water samples and Food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4, 5). For food samples, follow appropriate techniques for sample collection and processing as per guidelines (6). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (7). 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.



M1393 HiCrome™ MM Agar

HiCromeVeg™ Freedom from BSE / TSE worries
Single Streak Rapid Differentiation Series

HiCrome™ MM Agar (M1393) is also available as HiCrome™ MM HiVeg™ Agar (MV1393) wherein all the animal origin nutrients have been replaced by vegetable based nutrients.

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Limitations

1. Due to nutritional variations, some strains may show poor growth.
2. Though most of the *Salmonella* produce H₂S, certain non H₂S producing *Salmonella* species may appear as colourless colonies.
3. Due to nutritional variations, some strains may show poor growth.
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 recommended temperature.

Quality Control

Appearance of Powder : Cream to yellow coloured, 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.

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

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Escherichia coli</i> (25922) (00013*)	50-100	luxuriant	≥50%	light blue
<i>Salmonella</i> Enteritidis (13076) (00030*)	50-100	luxuriant	≥50%	black centered
<i>Salmonella</i> Typhimurium (14028) (00031*)	50-100	luxuriant	≥50%	black centered
<i>Citrobacter freundii</i> (8090)	50-100	good-luxuriant	≥50%	colourless#
<i>Pseudomonas aeruginosa</i> (27853) (00025*)	50-100	good-luxuriant	≥50%	colourless
<i>Enterococcus faecalis</i> (29212) (00087*)	≥10 ³	inhibited	0%	-

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 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4, 5).

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. Greenwald R., Henderson R.W. and Yappaw S., 1991, J. Clin. Microbiol. 29:2354.
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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.
6. 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.
7. 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.