



HiCrome™ Coliform Agar, Modified

M1832

Intended Use:

Recommended as a selective medium for the simultaneous detection of *Escherichia coli* and thermotolerant coliforms in water, milk, dairy products and food samples.

Composition**

Ingredients	g / L
Peptone, special	8.000
Sodium chloride	1.000
Yeast extract	3.000
Potassium dihydrogen phosphate	0.200
Dipotassium hydrogen phosphate	0.600
Bile Salts	0.800
Magnesium sulphate	0.200
Chromogenic mixture	0.200
Agar	10.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24 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™ Coliform Agar Modified is a selective medium recommended for the simultaneous detection of *E.coli* and thermo-tolerant coliforms in water and food samples (1). Peptone special and yeast extract provide carbon, nitrogen compounds, long chain amino acids, vitamins and essential growth nutrients to the organisms. The phosphates buffer the medium well. Magnesium sulphate helps colour development. Bile salts inhibits gram-positive organisms. Sodium chloride maintains osmotic balance. The chromogenic mixture contains two chromogenic substrates, which enables the detection of two specific enzymes, β -galactosidase and β -glucuronidase. β -galactosidase produced by coliforms cleaves one chromogen, resulting in the pink colouration of coliform colonies. The enzyme β -glucuronidase produced by *E.coli*, cleaves X-glucuronide. *E.coli* forms dark blue to violet coloured colonies due to cleavage of both the chromogens (2,3,4). *E.coli* strains that are β -glucuronidase negative (serotype O157:H7) produce pink coloured colonies. Other gram negative bacteria able to grow at (44±0.5 °C) produce white or colourless colonies.

Transfer 1 ml of product to analyze its tenfold dilutions to sterile Petri plates. Pour 12 ml of medium, mix well and allow to solidify. Overlay with 4 ml of medium, allow to solidify and incubate at 43-45°C for 18-24 hours.

Type of specimen

Food and Dairy samples; Water samples.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6,7). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (8). 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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. β -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.
3. Further biochemical and serological test are necessary for confirmation.

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 beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium

Light yellow to yellow clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 2.4% 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 24 hours (48 hours if necessary).

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50-100	good	40-50%	colourless
<i>Enterobacter cloacae</i> ATCC 23355	50-100	good-luxuriant	≥50%	pink
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	≥10 ⁴	inhibited	0%	
<i>Klebsiella pneumoniae</i> ATCC 13883 (00097*)	50-100	good-luxuriant	≥50%	light pink
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	≥10 ⁴	inhibited	0%	
<i>Escherichia coli</i> ATCC 10536	50-100	good-luxuriant	≥50%	dark blue/violet
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	good-luxuriant	≥50%	dark blue/violet

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

References

1. Manafi M. and Kneifel W., 1989, Zentralbl. Hyg., 189:225.
2. Frampton E. W., Restaino L. and Blaszkowski N., 1988, J. Food Prot., 51:402.
3. Kilian M. and Bülow P., 1976, Acta. Pathol. Microbiol. Scand., Sect. B, 84:245.
4. LeMinor L. and Hamida F., 1962, Ann. Inst. Pasteur (Paris), 102:267.
5. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
6. 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.
7. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
8. Lipps WC, Braun-Howland EB, Baxter TE, eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
9. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
10. 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.

Revision : 04/2024

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.