



HiCrome™ Coliform Agar w/ SLS

M1300

Intended use

HiCrome Coliform Agar w/ SLS is a selective agar recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples.

Composition**

Ingredients	Gms / Litre
Peptone, special	3.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	3.000
Potassium dihydrogen phosphate	1.700
Sodium pyruvate	1.000
L-Tryptophan	1.000
Sodium lauryl sulphate	0.100
Chromogenic mixture	0.200
Agar	12.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27 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. Add 5mg/l novobiocin before autoclaving the medium, when a high number of gram positive accompanying bacteria are expected. Mix well and pour into sterile Petri plates.

Principle And Interpretation

HiCrome™ Coliform Agar w/ SLS is a selective medium recommended for the simultaneous detection of *Escherichia coli* and total coliforms in water and food samples (4). Peptone special and sodium pyruvate provide essential growth nutrients to the organisms. The phosphates buffer the medium well. The medium composition helps even the sublethally injured coliforms to grow rapidly. Sodium lauryl sulphate inhibits gram-positive organisms. The chromogenic mixture contains two chromogenic substrates. The enzyme β -galactosidase produced by coliforms cleaves one chromogen, resulting in the salmon red 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 (1,2,3). The addition of L-Tryptophan improves the indole reaction, thereby increasing detection reliability in combination with the two chromogens. To confirm *E. coli*, add a drop of Kovacs reagent (R008) on the dark-blue to violet colony. Formation of cherry-red colour indicates positive reaction.

Type of specimen

Food samples ; Water samples

Specimen Collection and Handling

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

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. Certain species of *Shigella* and *Salmonella* are β -glucuronidase positive

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 1.2% Agar gel.

Colour and Clarity of prepared medium

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

Reaction

Reaction of 2.7% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

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	Indole production
<i>Citrobacter freundii</i> ATCC 8090	50-100	good-luxuriant	≥50%	salmon to red	negative reaction
<i>Escherichia coli</i> ATCC 8739 (00012)*	50-100	good-luxuriant	≥50%	dark blue/violet	positive, confirmation of red colour around the colony by addition of Kovacs reagent (R008)
<i>Enterobacter cloacae</i> ATCC 23355 (00082*)	50-100	good-luxuriant	≥50%	salmon to red	negative reaction
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	≥10 ³	inhibited	0%		
<i>Klebsiella pneumoniae</i> ATCC 13883 (00097*)	50-100	good-luxuriant	≥50%	light pink	negative reaction
<i>Salmonella Enteritidis</i> ATCC 13076 (00030*)	50-100	good	40-50%	colourless	negative reaction
<i>Shigella flexneri</i> ATCC 12022 (00126*)	50-100	good	40-50%	colourless	negative reaction

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store dehydrated powder and prepared medium on receipt 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 (7,8).

Reference

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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.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
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Revision : 02 /2018

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