



Technical Data

HiCrome™ Chromogenic Coliform agar (CCA Agar)

M1991I

Intended Use

Recommended for detection of *Escherichia coli* and coliforms in water samples. The composition and performance criteria of this medium are as per the specifications laid down in ISO 9308-1:2014.

Composition**

ISO 9308-1:2014 Specification -Chromogenic Coliform agar (CCA Agar)

Ingredients	g / L
Enzymatic digest of casein	1.000
Yeast extract	2.000
Sodium chloride	5.000
Sodium dihydrogen phosphate, 2H ₂ O	2.200
Disodium hydrogen phosphate	2.700
Sodium pyruvate	1.000
Sorbitol	1.000
Tryptophan	1.000
Tergitol-7	0.150
6-chloro-3-indoxyl β-D-galactopyranoside	0.200
5-bromo-4-chloro-3-indoxyl- β-D-glucuronic acid cyclohexyl ammonium salt, monohydrate (X-beta-G-glucuronide CHX salt)	0.100
IPTG (Isopropyl-β-D-thiogalactopyranoside)	0.100
Agar	9.0 to 18.00
Final pH (at 25°C)	6.8±0.2

M1991I - HiCrome™ Chromogenic Coliform agar (CCA Agar)

Ingredients	g / L
Tryptone #	1.000
Yeast extract	2.000
Sodium chloride	5.000
Sodium dihydrogen phosphate, 2H ₂ O	2.200
Disodium hydrogen phosphate	2.700
Sodium pyruvate	1.000
Sorbitol	1.000
Tryptophan	1.000
Tergitol-7	0.150
6-chloro-3-indoxyl β-D-galactopyranoside	0.200
5-bromo-4-chloro-3-indoxyl- β-D-glucuronic acid cyclohexyl ammonium salt, monohydrate (X-beta-G-glucuronide CHX salt)	0.100
IPTG (Isopropyl-β-D-thiogalactopyranoside) Agar	0.100
Final pH (at 25°C)	15.000
	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Enzymatic digest of casein

Directions

Suspend 30.92 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE. DO NOT OVERHEAT.** Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

HiCrome™ Chromogenic Agar is a selective medium recommended by ISO for enumeration of *Escherichia coli* and coliform bacteria (1). The medium contains three chromogenic substrates. The enzyme β-D-galactosidase produced by coliforms cleaves 6-chloro-3-indoxyl 1-β-D-galactopyranoside to form pink to red coloured colonies (1). The enzyme β-D-glucuronidase produced by *E.coli*, cleaves 5-bromo-4chloro-3-indoxyl 1-β-D-glucuronic acid (1) Colonies of *E.coli* give dark blue to violet coloured colonies due to cleavage of both the chromogens. The presence of the third chromogen IPTG enhances the colour reaction. Addition of L-Tryptophan improves the indole reaction thereby increasing the detection reliability.

Tryptone, sodium pyruvate and sorbitol provide nitrogenous substances, fermentable carbohydrate and other essential growth nutrients for the organisms. Phosphates buffer the medium. The media formulation helps even sub-lethally injured coliforms to recover and grow rapidly. Tergitol-7 inhibits gram-positive as well as some gram-negative bacteria other than coliforms (1). The medium is inoculated either by pour plate technique or by spreading the sample on the surface of plated medium. Membrane filter technique can also be used. To confirm *E.coli*, add a drop of Kovacs reagent on the dark blue to violet colony. Formation of cherry red colour indicates a positive reaction.

Type of specimen

Water samples.

Specimen Collection and Handling:

Processing (1)

Filtration:

Filter 100ml of the sample using membrane filter. The minimum volume for filtration should be 10ml (or dilution) so that to ensure even distribution of the bacteria on the membrane filter.

Incubation and differentiation:

After filtration place the membrane filter on HiCrome™ Chromogenic Coliform agar (CCA Agar), ensuring that no air is trapped underneath, invert petri dish and incubate at $36^{\circ}\text{C} \pm 2$ for 21 ± 3 hours. Examine the colony on membrane filters for color change.

Confirmation : Biochemical and serological tests are performed for confirmation.

Warning and Precautions

Read the label before opening the container. The media should be handled by trained personnel only. 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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
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

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

Light yellow coloured opalescent gel forms in Petri plates

Reaction

Reaction of 3.09% w/v aqueous solution at 25°C . pH : 6.8 ± 0.2

pH

6.60-7.00

Cultural Response

Productivity: Cultural response observed after an incubation at $36^{\circ}\text{C} \pm 2$ for 21 ± 3 hours. Recovery rate is considered as 100% for bacteria growth on Reference medium - Soyabean Casein Digest Agar (Tryptone Soya Agar).

Selectivity: Cultural response observed after an incubation at $36^{\circ}\text{C} \pm 2$ for 21 ± 3 hours.

Specificity: Cultural response observed after an incubation at $36^{\circ}\text{C} \pm 2$ for 21 ± 3 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony#
Productivity				
<i>Escherichia coli</i> ATCC 25922 (00013)*	50-100	luxuriant	$\geq 70\%$	dark blue to violet
<i>Escherichia coli</i> ATCC 8739 (00012)*	50-100	luxuriant	$\geq 70\%$	dark blue to violet
<i>Citrobacter freundii</i> ATCC 43864 (00006)*	50-100	luxuriant	$\geq 70\%$	pink to red

## <i>Klebsiella aerogenes</i> ATCC 13048 (00175)*	50-100	luxuriant	$\geq 70\%$	pink to red
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Selectivity

<i>Enterococcus faecalis</i> ATCC 19433 (00009)*	$\geq 10^4$	inhibited
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Specificity

<i>Pseudomonas aeruginosa</i> ATCC 10145 (00024)*	10^3 - 10^4	growth	-	colourless
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Key * : Corresponding WDCM numbers # : either on plate or membrane

Formerly known as *Enterobacter aerogenes*

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 (2,3).

Reference

1. International Organization for Standardization. Water quality: Enumeration of *E.coli* and coliform bacteria. Part I- Membrane filtration methods for bacteria with low bacterial background flora. ISO 9308-1:2014.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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.

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Disclaimer :

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