



HiCrome™ E. coli Agar

M1295I

Intended Use:

Recommended for the detection and enumeration of *Escherichia coli* in foods without further confirmation on membrane filter or by indole reagent. The composition and performance of this media are as per specifications laid down in in ISO 16649-2:1999. It can also be used to isolate *Escherichia coli* from clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptone	20.000
Bile salts mixture	1.500
X-Glucuronide	0.075
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36.57 grams 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™ E.coli Agar is based on Tryptone Bile Agar to detect *Escherichia coli* in foods (1), where recovery of *E.coli* is faster, more reliable and accurate. Most of the *E.coli* strains can be differentiated from other coliforms by the presence of enzyme glucuronidase, which is highly specific for *E.coli* (2). The chromogenic agent X-glucuronide used in this medium helps to detect glucuronidase activity of *E.coli*. *E.coli* cells absorb X-glucuronide and the intracellular glucuronidase enzyme splits the bond between the chromophore and the glucuronide. The released chromophore gives bluish green colouration to the *E.coli* colonies. The formulation is in accordance with ISO 16649-2 (3). It can also be used to isolate *E.coli* from clinical samples

Tryptone provides carbon, nitrogen compounds, long chain amino acids, vitamins and other essential growth nutrients to the organisms. Bile salts mixture inhibits gram-positive organisms. The surface of the plated medium is dried before use. Dilute food samples 1:5 or 1:10 with 0.1% (w/v) sterile Peptone Water (M028) and homogenize in a blender or a stomacher. Pipette 0.5 ml or 1.0 ml of the homogenized food sample on to the plate and spread with sterile glass spreader. Incubate the plates at 30°C for 4 hours and then at 44°C for 18 hours.

Type of specimen

Clinical samples - stool, urine , 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).

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.

Limitations

1. β -glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative.
2. Certain species of *Salmonella* are β -glucuronidase positive.
3. Some species may show poor growth due to nutritional variations.

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, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.66% 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 44°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	≥50%	bluish green
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50-100	luxuriant	≥50%	colourless
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	≥10 ⁴	inhibited	0%	

Key : *Corresponding WDCM numbers.

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 (5,6).

Reference

1. Anderson J.M. and Baird-Parker A.C., (1975), J. Appl. Bact., 39:111.
2. Hansen W. and Yourassawsky E., (1984), J.Clin. Microbiol., 20:1177.
3. International Standard ISO 16649-2: 1999. Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of β -glucuronidase-positive *Escherichia coli*.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition
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.



In vitro diagnostic medical device



CE Marking



Storage temperature



Do not use if package is damaged



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