HiCrome™ ECC HiVeg™ Agar

MV1293

Intended Use:

Recommended for presumptive identification of Escherichia coli and other coliforms in food and environmental samples.

Composition**

Ingredients	g / L
HiVeg™ special peptone	5.000
Yeast extract	3.000
Lactose	2.500
Disodium hydrogen phosphate	3.500
Potassium dihydrogen phosphate	1.500
Sodium chloride	5.000
Chromogenic mixture	20.300
Neutral red	0.030
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 55.83 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

Escherichia coli, a member of the family *Enterobacteriaceae* is a part of normal flora of the intestinal tract of humans and a variety of animals. Although most of *E.coli* does not cause gastrointestinal illnesses, certain groups of *E.coli* can cause life-threatening diarrhoea and sever sequelae or disability (1).

HiCrome™ ECC Agar is a differential medium recommended for the presumptive identification of *E.coli* and other coliforms in food and environmental samples (2). HiCrome™ ECC HiVeg™ Agar is prepared by completely replacing animal peptones with vegetable peptone to avoid BSE/TSE risks. The medium contains two chromogens. One of the chromogen is cleaved by the enzyme glucuronidase produced by *E.coli* to give blue to purple coloured colonies whereas the other chromogen is cleaved by the enzyme galactosidase, produced by majority of coliforms, resulting in the formation of rose-pink coloured colonies (3,4).

HiVeg™ special peptone, yeast extract provide nitrogenous, carbonaceous substances, long chain amino acids, vitamin B complex and other essential growth nutrients. Lactose is the fermentable carbohydrate, which aids in detecting lactose fermenters with neutral red as an indicator. Disodium hydrogen phosphate and potassium dihydrogen phosphate buffers the medium well. Sodium chloride maintains the osmotic equilibrium. Dry the surface of plate medium.

Dilute the food sample by 1:5 or 1:10 with 0.1% sterile Peptone HiVeg™ Water (MV028) and homogenize in a blender or a stomacher. Spread 0.5 ml or 1.0 ml of the homogenate over the agar surface with a sterile glass spreader and incubate the plates at 37°C for 18-24 hours. Count the blue/purple colonies and multiply with the dilution factor. The number of *E.coli* is reported per gram of food. The medium should be used only for in-vitro diagnostic purpose. Wear mask while handling the dehydrated product and avoid contact with eyes.

Type of specimen

Food and environmental samples.

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).

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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. ß-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.

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 pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Reddish pink coloured, opaque gel forms in Petri plates

Reaction

Reaction of 5.58% 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 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=70%	blue/purple
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant	>=70%	straw
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	>=70%	rose/pink
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	luxuriant	>=70%	pink

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 (6,7).

Reference

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5.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
6.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
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