HiCrome™ Rapid ECC Broth

Recommended for rapid detection of *Escherichia coli* and other *Enterobacter*ioaceae from water samples.

M2011

Composition **

Ingredients	Grams/Litre
Peptone special	24.00
Sodium chloride	5.00
Disodium hydrogen phosphate	1.00
Sodium thiosulphate	5.00
Ferric citrate	1.00
Lactose	5.00
Phenol red	0.018
Selective mix	1.50
Chromogenic substrate	3.83

Final pH (at 25°C) 7.4 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.35 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Mix well and dispense into sterile tubes or flasks as desired.

Principle and Interpretation

HiCrome™ Rapid ECC Broth is designed for detection and confirmation of *Escherichia coli* and other coliforms from water samples. The major microbial water contaminants are coliforms include *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella, Citrobacter, Vibrio*, and *Pseudomonas* (1). This test was designed for the rapid detection and differentiation of these organisms.

Peptone special provides nitrogen and carbon source, long chain amino acids, vitamins and other essential growth nutrients. Phosphates buffer the medium. Lactose is the fermentable carbohydrate and phenol red is the indicator. Lactose fermenting organisms gives yellow colour to the medium while lactose non-fermentors gives pink to red colour. The chromogenic substrate is used to detect the presence of ß-D-glucuronidase produced by *E.coli* thus imparting blue colour to the medium. However since *E.coli* also ferments lactose, the presence of *E.coli* is indicated by bluish gren to green colour. The detection of H_2S production is enhanced by the presence of specific H_2S detectors. The medium turns black in case of H_2S producers such as *Salmonella*, *Citrobacter* etc are present. The phosphate salts provide buffering action for rapid growth of coliforms. Sodium chloride helps to maintain the osmotic balance. Selective mix present in the medium suppresses the growth of gram positive microorganisms. Recovery of these pathogens is faster and reliable.

Type of specimen

Water and waste water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1, 2, 3).

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. ß-glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative.
2. ß-glucuronidase negative *E.coli* will impart yellow colour to the medium.
3. Further confirmation must be carried out for E.coli by indole test.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the recommended temperature.

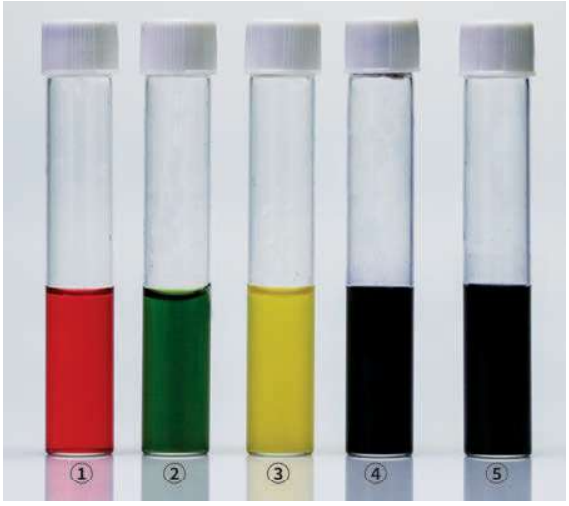
Quality Control

Appearance of Powder : Light yellow to pink homogeneous free flowing powder

Colour and Clarity : Red coloured clear solution in tubes

Reaction : Reaction of 4.63% w/v aqueous solution at 25°C. pH : 7.4±0.2

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 12-18 hours.



1. Control
2. *Escherichia coli* ATCC 25922 (00013*)
3. *Klebsiella pneumoniae* ATCC (13883) (00097*)
4. *Citrobacter freundii* ATCC 8090
5. *Salmonella* Typhimurium ATCC 14028 (00031*)

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Organism (ATCC)	Inoculum (CFU)	Growth	Colour change in medium
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	green
Klebsiella pneumoniae ATCC (13883) (00097*)	50-100	luxuriant	yellow
Citrobacter freundii ATCC 8090	50-100	luxuriant	black
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	black
Enterococcus faecalis ATCC 29212 (00087*)	>=10^3	inhibited	
Staphylococcus aureus subsp *aureus* (25923) (00034*)	>=10^3	inhibited	

Key : * = corresponding WDCM Numbers

Storage and Shelf-life

Store dehydrated powder and prepared medium at 2-8°C in tightly closed container. On opening, product should be properly stored dry, after tightly capping the bottle inorder 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4, 5).

References

1. Methods for Examination of Waters and Associated Materials, Environment Agency, 1998, Standing Committee of Analysts.
2. Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st ed., APHA, Washington, D.C.
3. 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.
4. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 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.