HiCrome PA Broth

M1663

HiCrome PA Broth is recommended for the detection of presence and absence of coliform bacteria in water.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
Lactose	5.000
Bile salts mixture	1.500
Dipotassium hydrogen phosphate	3.000
Potassium dihydrogen phosphate	1.500
Sodium chloride	5.000
2-Nitrophenyl ß-D-galactopyranoside(ONPG)	1.250
4-methylumbelliferyl ß-D-glucuronide(MUG)	0.100
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.35 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 50°C. Dispense into sterile test tubes.

Principle And Interpretation

Examination of water for the presence of marker groups such as coliforms is one of the most common tests in food microbiology laboratory, partly because of the relative ease and speed with which these tests can be accomplished. Where it is claimed that water has been processed for safety, the finding of such organism demonstrates a failure of the process (1) HiCrome PA Broth is a modification of the medium originally devised by Hajna and Perry (2) and is used for the detection of presence and absence of coliform bacteria in water.

The fluorogenic compound 4-Methylumbelliferyl ß-D-glucuronide (MUG) is incorporated in the medium for the fluorogenic detection of *Escherichia coli* , the main indicator organism for the faecal contamination of water. The enzyme ß-glucuronidase possessed by *Escherichia coli* hydrolyses MUG to yield a fluorescent end product 4-Methylumbelliferone; which can be detected when the medium is observed for fluorescence under UV light (3,4) MUG also detects anaerogenic strains which may not be detected in the conventional procedure (3). ONPG test is used to determine the presence or absence of ß-galactosidase in organisms (5) and is also important in differentiating *Enterobacteriaceae* which are commonly classifed according to their ability to ferment lactose. ONPG is similar in structure to lactose. The presence of two enzymes, permease and ß-D-galactosidase are required to demonstrate lactose fermentation. True lactose nonfermenters do not possess either of these enzymes. Late lactose fermenting organisms do not have permease but do possess ß-galactosidase. If ß-galactosidase is present, the colourless ONPG is split into galactose and o-nitrophenol, a yellow compound (6).

Casein enzymic hydrolysate provides essential nutrients. Lactose is the fermentable carbohydrate, sodium choride maintains osmotic equilibrium. The medium has a strong buffering system to control the pH in the presence of fermentative action. Bile salts inhibit gram-positive bacteria especially *Bacillus* species and faecal Streptococci. Mostly ß-glucuronidase activity occurs within 4 hours but some weakly ß-glucuronidase positive strains require overnight incubation (7).

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light amber coloured, clear solution without any precipitate

Reaction

Reaction of 3.7% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
M1663: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	ONPG	Fluorescence at 366nm
Escherichia coli ATCC 25922	50-100	luxuriant	positive reaction,yellow colour	positive, throughout the tube
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	positive reaction,yellow colour	negative
Klebsiella pneumoniae ATCC 13883	50-100	luxuriant	positive reaction,yellow colour	negative
Proteus mirabilis ATCC 25933	50-100	luxuriant	negative reaction, no yellow colour or colourless	negative
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	negative reaction, no yellow colour or colourless	negative
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	negative reaction, no yellow colour or colourless	negative
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	negative reaction, no yellow colour or colourless	negative

Storage and Shelf Life
Store dehydrated powder and prepared medium at 2-8°C. Use before expiry period on the label.

Reference
1.Corry J. E. L., Curtis G. D. W., and Baird R. M., Culture Media For Food Microbiology, Vol. 34, Progress in industrial Microbiology, 1995, Elsevier, Amsterdam
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4.Robinson B. J., 1984, Appl. Environ. Microbiol., 48:285.
5.MacFaddin J. F, 2000, Biochemical Tests for Identification of Medical Bacteria. 3rd Ed. Philadelphia, Lippincott Williams and Wilkins, p. 160-9.
6.Isenberg H. D., (Eds.), Clinical Mirobiology Procedures Handbook, Vol. I, Washington D.C. American Society for Microbiology; 1992, p.l. 19.20-1.19.22.
7.Eaton A. D., Clesceri L. S. and Greenberg A. W.,(Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C

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