



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
WATER

Axel Dellenbusch
Marketing Manager Microbiology
EMD Chemicals (formerly EM Science)
480 South Democrat Road
Gibbstown, NJ 08027-1297

Dear Mr. Dellenbusch:

EPA's Office of Ground Water and Drinking Water (OGWDW) in conjunction with EPA's Statistics and Analytical Support Branch (SASB) has reviewed EMD Chemicals' request for the approval of Fluorocult LMX broth as an acceptable version of the ReadyCult® Coliforms Presence/Absence Test Method. OGWDW and SASB have determined that Fluorocult LMX (ATP case No. BD04-0001) is an acceptable version of the ReadyCult® test method. Therefore, Fluorocult LMX broth may be used to monitor for total coliforms and *E. coli* under the Total Coliform Rule (40 CFR 141.21). This approval is contingent on the current instructions (as of the date of this letter) for the use of Fluorocult LMX broth and medium formulation, and any modification to the current instructions or formulation may require additional review by OGWDW and SASB. Please see the attachment to this letter.

We appreciate EMD Chemicals' continued interest in the development of environmental compliance monitoring methods. If you have any questions regarding our determination, please contact Robin K. Oshiro (SASB) at 202/566-1075, or Herb Brass (OGWDW) at 513/569-7936 at your convenience.

Sincerely,

William A. Telliard, Director
Director of Analytical Methods,
Engineering and Analysis Division (4303-T)

Herb Brass, Ph.D.
Technical Support Center (MS-140)
Office of Ground Water and Drinking Water

cc: USEPA Regional Administrators (all Regions)
Quality Assurance Managers (all Regions)
Water Management Division Directors (all Regions)
ATP Coordinators (all Regions)
Gregory Carroll, USEPA, OGWDW/TSC
James Sinclair, OGWDW/TSC
Lillian Holmes, USEPA, OGWDW/TSC
Maria Gomez-Taylor, USEPA, EAD
Paul Berger, USEPA, OGWDW
Kevin Roberts, CSC, SCC
James Boiani, CSC, SCC
Yildiz Chambers, CSC, Biology Studies Group

Enrichment for the simultaneous detection of total coliforms and E.coli in water, food and dairy products by the fluorogenic procedure.

Mode of Action

LMX Broth first described by MANAFI and KNEIFEL (1989) was modified by MANAFI and OSSMER (1993) to improve the substrate utilization, to increase sensitivity and at the same time reduce the overall incubation time to 24 hours.

Fluorocult® LMX Broth Modified contains phosphate buffer to guarantee a high growth rate for total coliforms. Lauryl sulfate largely inhibits the accompanying Gram-positive flora. By adding the chromogenic substrate 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside, which is cleaved by coliforms and the fluorogenic substrate 4-methylumbelliferyl- β -D-glucuronide, which is highly specific for E.coli, the simultaneous detection of total coliforms and E.coli is possible. A color change of the broth from yellow to blue-green indicates the presence of coliforms. In addition a blue fluorescence under long-wave UV light permits the rapid detection of E.coli. As tryptophan is added to the broth, the indole reaction is easily done by adding Kovacs reagent. The formation of a red ring additionally confirms the presence of E.coli. The enzyme synthesis is amplified by 1-isopropyl- β -D-1-thio-galactopyranoside and increases the β -D-galactosidase activity.

Typical composition (g/Liter)

Tryptose 5.0; sodium chloride 5.0; sorbitol 1.0; tryptophan 1.0; dipotassium hydrogen phosphate 2.7; potassium dihydrogen phosphate 2.0; lauryl sulfate sodium salt 0.1; 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-GAL) 0.08; 4-methylumbelliferyl- β -D-glucuronide (MUG) 0.05; 1-isopropyl- β -D-1-thio-galactopyranoside (IPTG) 0.1.

Preparation

Food testing:

Suspend 17 g (single strength) in 1 liter of purified water. Heat to boiling to dissolve completely. Fill up to 20 ml aliquots into tubes. Autoclave for 15 min. at 121°C.

Water testing:

If 100 ml water samples (e.g. drinking water) are to be tested, suspend 34 g (double strength) in 1 liter of purified water. Heat to boiling to dissolve completely. Transfer 100 ml aliquots into bottles (250 ml capacity). Autoclave for 15 min. at 121°C.

pH: 6.8 \pm 0.2 at 25 °C.

The prepared broth is clear and yellowish-brown.

Experimental Procedure and Evaluation

Application varies with the method/samples used for water or food testing

Incubation: 24 hours at 35 \pm 0.5°C aerobically.

Interpretation of results

Total coliforms: broth color has changed to blue-green

E.coli: blue-green color of the broth and blue fluorescence using long-wave UV light source (366 nm). Overlay with Kovacs reagent for the indole reaction – a red ring additionally confirms the presence of E.coli.

Note: if the fluorescence is negative after 24 hours of incubation **do not** add Kovacs reagent to check the indole reaction at this point. Kovacs reagent is an alcoholic solution which destroys the growth conditions in the broth. **Continue incubation for another 24 hours** followed by checking fluorescence and indole reaction.

Additives

EMD Cat. No.	Product	Pack size
1.13203.0001	UV lamp (366 nm)	1 ea
1.09293.0100	Kovac's Indole reagent	100 ml
1.11350.0002	Bactident® Indole (dropper bottle)	30 ml

Quality Control

Test strains	Color change to blue-green	fluorescence	Indole reaction
Escherichia coli ATCC 25922	+	+	+
Klebsiella pneumoniae ATCC 13883	+	-	
Enterobacter cloacae ATCC 13047	+	-	-
Citrobacter freundii ATCC 6750	+	-	
Citrobacter freundii ATCC 8090	+	-	
Shigella flexneri ATCC 12022	-	-	
Salmonella typhimurium ATCC 14028	-	-	

Literature

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- MANAFI, M., KNEIFEL, W.: Ein kombiniertes Chromogen-Fluorogen-Medium zum simultanen Nachweis der Coliformengruppe und von E.coli in Wasser. – **Zbl. Hygiene und Umweltmedizin** **189**; 225-234 (1989)
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