

An evaluation of a new chromogenic / flourogenic microbiology media system for detection of total coliforms and *E. Coli* in water

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Abstract

Readycult® Coliforms (RC), containing 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal) and 4-methylumbelliferyl- β -D-glucuronide (MUG), is a new selective and differential medium for determination of the presence or absence of total coliforms (TC) and *E. coli* in drinking and surface water. After incubation at 37°C for 24h, the development of blue-green in an initially colourless solution is specific for total coliforms; fluorescence at 366 nm in the same vessel demonstrates the presence of *E. coli*. This method was evaluated with 300 samples, using 100-ml sample volumes, and compared to accepted methods by the E.P.A. and DIN. A total of 90 (62 *E. coli*) confirmed vessels were positive by the E.P.A test, 94 (60 *E. coli*) by the DIN test and 100 (63 *E. coli*) by the RC system. False-positive results for coliforms and *E. coli* were by the EPA test 1(1), by the DIN test 1 (2) and by the RC system 3 (0). *Aeromonas hydrophila* strains showing false-positive results with RC system. With the EPA method one *E. coli* strain and with the DIN method two *E. coli* strains could not be detected. The Readycult® system was found to be as sensitive as Standard Methods and simultaneously enumerated *E. coli* and coliforms in the same analysis. The efficiency and rapidity of the detectable reactions make RC system a very useful tool in routine water microbiology.

Introduction

Because of the potential consequences of waterborne diseases, microbial contamination is still considered to be the most critical risk factor in drinking water quality. The WHO Guidelines for Drinking-water Quality (WHO, 1993) states that it is impractical to monitor drinking water for every possible microbial pathogen. Monitoring the microbiological quality of drinking water relies largely on examination for indicator bacteria, such as coliforms, *E. coli* and enterococci using membrane filtration technique (MF), the multiple-tube fermentation test (MPN), and the presence-absence test (P/A). MPN and P/A tests require a minimum of 2 days, followed by another 2 days for the confirmation. There is a need for rapid methods, particularly in emergencies, to quickly determine the indicator bacteria and pathogens in water. As both, coliforms and *E. coli* are still important indicators of water pollution, there arises the necessity to create media which are able to detect both bacteria. This would then guarantee a better performance of microbiological quality control. Several attempts have been made to simultaneously detect coliforms and *E. coli* and novel methods have been introduced, based on the detection of β -D-galactosidase (β -GAL) and β -D-glucuronidase (GUD) using fluorogenic and/or chromogenic substrates (Manafi, 1996).

Readycult® Coliforms (RC, Merck) is a new selective and differential medium for detecting the presence/absence of total coliforms (TC) and *E. coli* in drinking and/or surface water. This medium, containing 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-GAL) and 4-methylumbelliferyl- β -D-glucuronide (MUG), is presented as a pre-dispensed, sterile granulated medium, which can be directly added to the water sample. MUG is hydrolyzed by GUD yielding 4-MU, which shows blue fluorescence under UV light (366 nm) indicating the presence of *E. coli*. The activity of the enzyme β -D-galactosidase produces a blue-green coloration in the water samples, thereby indicating the presence of coliforms and *E. coli*.

Material and Methods

A total of 300 drinking and surface water samples were collected from different sources in Austria. All samples were transported in an icebox and bacteriological analysis was initiated immediately.

Two internationally used standards procedures (the DIN procedure and the E.P.A. procedure) and the Readycult® system were used to recover coliforms and *E. coli* in each water sample (**FIG. 1**).

Readycult® method is a one-step, ready-to-use, granulated medium to which a water sample is added (**FIG. 2, see pictures**). The contents of one snap-pack is added to a 100 ml water sample, mixed, and incubated at 37°C for 24h. Blue-green coloration in an initially colourless

solution is specific for total coliforms; fluorescence at 366 nm in the same vessel demonstrates the presence of *E. coli*. To confirm presence of *E. coli* indole production can be demonstrated using KOVACS' indole reagent. All positive bottles were subcultured by streaking on Eosin methylene blue lactose sucrose agar (EMB) from Merck. The organisms were isolated and identified using API 20E test strips.

Results

The comparison of tested methods for detecting *E. coli* and coliforms in water samples and number of confirmed positive and false-positive samples are presented in TABLE I. Out of 300 samples, a total of 91 (63 *E. coli*) presumptive vessels were positive by the EPA test, 95 (60 *E. coli*) by the DIN test and 103 (63 *E. coli*) by the RC system. On the other hand, a total of 90 (62 *E. coli*) confirmed vessels were positive by the EPA test, 94 (60 *E. coli*) by the DIN test and 100 (63 *E. coli*) by the RC system. False-positive results for coliforms/*E. coli* were 1/1 by the EPA test, 1/2 by the DIN test and 3/0 by the RC system. The false-positive results were *Aeromonas hydrophila* strains. With the EPA method one *E. coli* strain and with the DIN method two *E. coli* strains could not be detected.

Discussion

Augoustinos et al. (1993) isolated 95,7% GUD positive *E. coli* from a MUG medium and found additional *Shigella* and *Salmonella* strains capable of producing the GUD enzyme. Alonso et al. (1996) found some strains of *Klebsiella oxytoca*, *Serratia fonticola* and *Yersinia intermedia* capable of GUD production. It should be mentioned, that some strains of flavobacteria, staphylococci, streptococci and clostridia are also known to produce GUD. Growth of many of these micro-organisms, however, can be inhibited by selective agents in the media or by the incubation conditions (Gauthier et al., 1991, Manafi, 1995).

Media containing X-Gal are already used in water monitoring (Jermini et al. 1994, Manafi 1995, 1996). It was found that coliform strains produced sharp blue colonies on the agar plate, because of the insolubility of indigo dye, which does not alter the viability of the colonies. X-Gal was also a faster and more sensitive parameter for total coliforms than gas production from lactose (Ossmer 1993). Another medium, Merck's Fluorocult® LMX Broth (X-Gal/MUG), was evaluated as a multiple-tube fermentation test (Betts et al. 1994) and as Presence/Absence (P/A) test (Lee et al. 1995, Manafi 1995, Fricker and Fricker 1996). An evaluation of a number of P/A tests for coliforms and *E. coli*, including Fluorocult® LMX Broth (Merck) and Colilert (Idexx) has been published under the Department of the Environment series in the UK (Lee et al., 1995). They compared four P/A tests with UK Standard methods and found that more coliforms were detected than with membrane filtration technique; in addition, it was shown that

results in Fluorocult® LMX Broth were the easiest to interpret. The study concludes that there is no P/A test that is best at all locations for both, coliforms and *E. coli*, because of possible ecological differences between sources. It is important that particular P/A tests are validated in each geographical area before use.

Fricker and Fricker (1996) made an evaluation of Colilert system and Fluorocult® LMX Broth for their ability to recover *E. coli* and coliforms. Both methods gave similar recoveries for both groups of organisms. The Fluorocult® LMX system gave a number of false positive results, largely due to the presence of *Aeromonas* spp. Another study by Landre et al (1997) reported false positive coliform reaction in the Colilert system caused by *Aeromonas*. Data obtained, clearly demonstrate that *A. hydrophila* can elicit a positive coliform type reaction at very low densities. Cell suspensions as low as 1 cfu / mL were observed to yield a positive reaction. Similar results were obtained with other members from the mesophilic group of Aeromonads. Use of Colilert for monitoring water quality will lead to an over estimation of coliforms because *Aeromonas* is known to be present in treated drinking water supplies. In another study, testing 771 gram-negative bacterial strains with Fluorocult® LMX Broth, 98% of *E. coli*, isolated from drinking water, were β -glucuronidase positive. 98% of *E. coli* strains showed β -D-galactosidase activity and 99% gave a positive indole reaction (Manafi 1995). Only a few strains of *Vibrio metschnikovii*, *V. vulnificus*, *Aeromonas hydrophila* and *A. sobria* gave a false positive reaction with X-Gal.

In the present study, only a few non-coliform bacteria such as strains of *A. hydrophila* gave false-positive reactions with X-Gal. This is in accordance with Ley et al. (1993), who found that X-Gal medium, in addition to providing a rapid test for coliforms, also detected β -galactosidase-positive aeromonads and non-sheen-forming members of the *Enterobacteriaceae* on m-Endo Agar. They reported the low sensitivity of m-Endo for detecting *Aeromonas*, which is considered ubiquitous waterborne organisms and should not be present in drinking water (Moyer 1987). Solving this problem, Alonso et al. (1996) suggested adding Cefsulodin at 5 to 10 μ g/ml to the medium to inhibit growth of *Aeromonas* and *Flavobacterium* species. Geissler et. al. (1998) compared the performance of Fluorocult® LMX® Broth, Chromocult® Coliform-Agar (CC) and Chromocult® Coliform-Agar plus cefsulodin (10 μ g/mL) (CC-CFS), with *Standard Methods* multiple tube fermentation (MTF) for the enumeration of coliforms and *E. coli* from marine recreational waters. The traditional MTF was less sensitive for *E. coli* enumeration. Background interference was reduced on CC-CFS and the total coliform counts were more accurately .

Our results showed that Readycult® detected coliforms and *E. coli* in significantly more samples within 24 hours than the Standard Methods. Readycult® system showed false-positive results for *Aeromonas* spp., which are ubiquitous waterborne bacteria. The detection of coliforms and

E. coli with Readycult® is reduced to a total of 1 day, compared to 4 days by EPA method and 3 days by DIN method. The Readycult® system is as sensitive as Standard Methods and it simultaneously enumerated *E. coli* and coliforms in the same analysis. The efficiency and speed of the detectable reactions make this medium a very useful tool in routine water microbiology.

FIG. 1: VARIOUS PROTOCOLS USED TO DETECT COLIFORMS AND *E. COLI* IN WATER

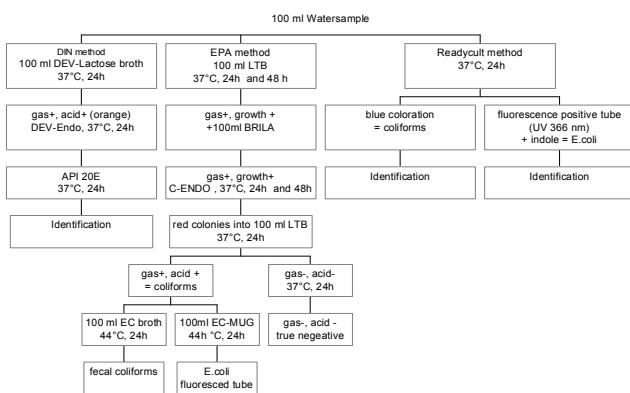


TABLE I. THE COMPARISON OF TESTED METHODS FOR DETECTING *E. COLI* AND COLIFORMS IN WATER. SAMPLES, NUMBER OF CONFIRMED POSITIVE AND FALSE POSITIVE SAMPLES^A.

Methods	presumptive		confirmed		false- positive	
	Coliforms	<i>E. coli</i>	Coliforms	<i>E. coli</i>	Coliforms	<i>E. coli</i>
EPA	91	63	90	62	1 ^b	1
DIN	95	60	94	60	1 ^b	2
RC	103	63	100	63	3 ^c	0

^a strains grown on EMB were identified by Gram stain, oxidase test and API 20E System

^b Api test was not detectable

^c false positive results with strains of *A. hydrophila*

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