Multiparametric comparison of chromogenic-based culture methods used to assess the microbiological quality of drinking water and the mFC method combined with a molecular confirmation procedure

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ABSTRACT

MI agar and Colilert®, as well as mFC agar combined with an *Escherichia coli*-specific molecular assay (mFC + *E. coli* rtPCR), were compared in terms of their sensitivity, ease of use, time to result and affordability. The three methods yielded a positive *E. coli* signal for 11.5, 10.8, and 11.5% of the 968 well water samples tested, respectively. One hundred and thirty-six (136) samples gave blue colonies on mFC agar and required confirmation. *E. coli*-specific rtPCR showed false-positive results in 23.5% (32/136) of cases. In terms of ease of use, Colilert was the simplest method to use while the MI method provided ease of use comparable to all membrane filtration methods. However, the mFC + *E. coli* rtPCR assay required highly trained employees for confirmation purposes. In terms of affordability, and considering contamination rate of well water samples tested, the Colilert method and the mFC + *E. coli* rtPCR assay were at least five times more costly than the MI agar method. Overall, compared with the other two methods tested, the MI agar method offers the most advantages to assess drinking water quality.

Key words | Colilert®, *E. coli*, mFC agar, MI agar, rtPCR

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INTRODUCTION

In the Province of Québec, the *Programme d'accréditation des laboratoires d'analyse*, is administered by the *Centre d'expertise en analyse environnementale du Québec* (CEAEQ), which certifies private, municipal and institutional laboratories. Since 2001, thermotolerant coliforms detection has been recommended by the CEAEQ for the

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assessment of the microbiological quality of drinking water. Indeed, in the presence of these microorganisms, drinking water is potentially contaminated with pathogens, and thus inadequate for human consumption.

However, the mFC method is not totally specific and non-fecal origin microorganisms can be detected with this method as false-positive results. Consequently, in 2010 the CEAEQ proposed amending their guidelines and since 2013 has required the measurement of the presence of *Escherichia coli* rather than thermotolerant coliforms, as recommended in the United States and many European countries (*Standard Methods* 1998, 2005; Government of Quebec 2013).

To comply with this new guideline, water testing companies must validate a new procedure able to detect the presence of *E coli* rather than thermotolerant coliforms in drinking water. Many methods are available to detect the presence of *E. coli* in water with high variability in cost. However, many studies show that methods based on the detection of the β-D-glucuronidase enzyme (the majority) fail to detect 5 to 8% of *E. coli* strains because they do not express this enzyme (Feng & Hartman 1982; Moberg *et al.* 1988; Hartman 1989; Martin *et al.* 1993). The mFC method based on lactose hydrolysis and high temperature incubation detects these *E. coli* strains. Accordingly, Maheux *et al.* (2011) suggest that it might be more advantageous to use the mFC method combined with an *E. coli*-specific real-time polymerase chain reaction (PCR) assay to confirm the identity of the isolated colonies (in only 1 hour) in the context of very low occurrence of water contamination and the higher cost of the β-D-glucuronidase-based methods.

In this study, we compared the sensitivity, ease of use, time to result and affordability of two methods (Colilert® and USEPA Method 1604 on MI agar; USEPA 2002) both based on β-glucuronidase enzyme expression and approved by the USEPA to detect the presence of *E. coli* in water, as well as the new combination of methods (mFC + *E. coli* rtPCR) proposed by Maheux *et al.* (2011).

MATERIALS AND METHODS

Sample collection

During the summers of 2011 and 2012, 968 1-L raw well water samples from individual households were collected in the Québec City region (Canada). For statistical validity, regions presenting a high concentration of *E. coli*-contaminated well water samples were targeted for sample collection in the present study. Each well water sample was divided in 100-mL subsamples for simultaneous testing using standard microbiological methods mFC agar, USEPA Method 1604 on MI agar and Colilert® (see sections on membrane filtration and liquid culture methods). The identity of blue colonies recovered from mFC agar (maximum 10 per plate) was further confirmed by *E. coli*-specific rtPCR (Maheux *et al.* 2011; see sections on preparation of the DNA extract, rtPCR primers and rtPCR amplification).

Membrane filtration method

The membrane filtration method was performed according to Maheux *et al.* (2009a). Two 100-mL volumes were filtered on Millipore filters with a standard platform manifold. The first filter was incubated on MI agar (BD, Franklin Lakes, NJ, USA) for 24 h ± 2 h at 35.0 ± 0.5 °C and the second on mFC agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for 24 h ± 2 h at 44.5 ± 0.5 °C before determining colony counts and colour. Each preparation of MI agar and mFC agar plates was tested for performance using pure cultures of target and non-target microorganisms, as recommended by the USEPA microbiology methods manual (USEPA 1978). Tests to confirm the sterility of the filter membranes and buffer used to rinse the filtration apparatus were also performed (*Standard Methods* 2005).

Liquid culture method

For the detection of *E. coli* in water samples with the Colilert® method (Colilert; IDEXX Laboratories Canada Corp., Toronto, Ontario, Canada), preparation, validation, storage and handling steps were all performed according to the manufacturer's instructions. Briefly, one snap pack containing the Colilert reagent was dissolved in 100 mL of sample water. The solution was then added to a Quanti-tray®, sealed and incubated at 35.0 ± 0.5 °C for 24 h ± 2 h prior to the identification of *E. coli* and total coliform positive samples. *E. coli* presents a yellow coloration and fluorescence under UV light (λ = 365 nm), whereas total coliforms presents a yellow coloration. Positive and negative

controls using pure cultures of target and non-target microorganisms were performed as recommended by the manufacturer.

Preparation of the DNA extract

rtPCR amplifications were performed using a bacterial suspension adjusted to a 0.5 McFarland standard (Fisher Scientific Company, Ottawa, Ontario, Canada). The cells were lysed using the BD Diagnostics-GeneOhm Rapid Lysis kit as recommended by the manufacturer (BD Diagnostics-GeneOhm, Québec, Quebec, Canada).

rtPCR primers

The sequences of the rtPCR primers evaluated in this study are shown in Table 1. Oligonucleotide primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA).

rtPCR amplification

Briefly, 1 μL of the standardized lysed bacterial suspension was transferred directly to a 24-μL mixture of rtPCR containing 50 mM KCl, 10 mM Tris–HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl2, 0.4 μM of each primer, 0.2 μM probe, 200 μM of each deoxyribonucleoside triphosphate (GE Healthcare Bio-Sciences Inc., Baie d'Urfé, Quebec, Canada), 3.3 μg per μL of bovine serum albumin (BSA; Sigma-Aldrich Canada Ltd, Oakville, Ontario, Canada), 0.06 μ g/μL methoxsalen (Sigma-Aldrich Canada Ltd), 0.5U of *Taq* DNA polymerase (Promega, Madison, WI, USA), and TaqStart antibody (Clontech Laboratories, Mountain View, CA, USA). Decontamination of the rtPCR mixtures prior to rtPCR was achieved using the UV crosslinker Spectrolinker™ Model XL-1000 (Spectronics Corporation, Westbury, NY, USA; Maheux *et al.* 2009b). For each experiment, 1 μL of sterile water was added to the rtPCR mixture as a negative control. For *tuf* gene amplification, the rtPCR mixtures were subjected to thermal cycling (1 min at 95 °C and then 45 cycles of 2 s at 95 °C, 10 s at 58 °C, and 20 s at 72 °C) in a Rotor-Gene thermocycler (QIAGEN Inc., Mississauga, Ontario, Canada).

Statistical analysis

All individual results were recorded using Microsoft Excel 2010 software (Microsoft Corporation; Redmond, WA, USA) and the statistical analysis was performed using the SAS 9.3 program (SAS Institute Inc. 2011, Cary, NC).

All water samples were recorded as positive (1) or negative (0) for *E. coli*. McNemar's test was used to compare paired proportions with a 95% confidence interval. When the (two- sided) *p*-value was less than 0.05, it was concluded that there was a significant difference between both methods. For an overly conservative measure of agreement, Cohen's kappa coefficient was also used to measure the inter-rater agreement. Fleiss (1981) magnitude guidelines were used to characterize the κ values (>0.75 = excellent, 0.40 to 0.75 = fair to good, and <0.40 = poor).

RESULTS AND DISCUSSION

Sensitivity

The enzyme-based MI agar and Colilert® test methods as well as the mFC + *E. coli* rtPCR assay were first tested with 100-mL subsamples obtained from 968 1-litre well water samples collected in the Québec City region during the summers of 2011 and 2012. The three methods yielded an *E. coli* positive signal for 111 (11.5%), 105 (10.8%), and

Table 1 | Primer and probe set used in this study for PCR amplification

Genetic target	Primers and probes	Primers and probes sequence (5′→3′)	References
tuf	TEcol553	TGGGAAGCGAAAATCCTG	Maheux *et al.* (2009b)
	TEcol754	CAGTACAGGTAGACTTCTG	
	TEco573-T1-B1	TET[a]-AACTGGCTGGCTTCCTGG-BHQ-1[b]	Maheux *et al.* (2011)

111 (11.5%) of the 968 well water samples tested, respectively (Table 2). In 2011, Maheux *et al.* showed that the mFC agar method detected 5.5% to 6.8% more *E. coli* colonies than the Readycult and Colilert enzymatic culture-based methods, respectively. Following these results, they suggested that the combination of mFC + *E. coli* rtPCR could be more sensitive than the enzymatic culture-based methods. The results obtained in the present study suggest that this better ubiquity (ability to detect all or most *E. coli* strains) of mFC agar is not transposed into better sensitivity, as the detection of *E. coli*-contaminated well water samples was statistically equivalent (Table 3).

Ease of use

In terms of ease of use, Colilert was the simplest method to use. Indeed, unit-dosed packaging eliminates media preparation. Furthermore, there is no repeat testing due to clogged filters. Finally, its use does not require well-trained employees. The MI method, on the other hand, provides ease of use comparable to all membrane filtration methods. The medium must be prepared and quality control carried out on each batch. Employee training is also more important than the Colilert method. However, employees already using the membrane filtration equipment can easily use this method. The mFC + *E. coli* rtPCR assay presents the same advantages and disadvantages as the MI agar membrane filtration method. However, it requires highly trained employees to confirm the identity of a presumptive *E. coli* colony isolated on mFC by rtPCR. Furthermore, since the mFC agar method must be used in combination with the mEndo agar method to detect both *E. coli* and total coliforms in water, two membrane filtrations must be carried out for the same water samples, multiplying preparation time by two, quality control, sample treatment and the collection of results.

Time to result

In terms of time to result, the Colilert and MI methods both required 24 hours prior to obtaining results without the confirmation step, whereas the mFC + *E. coli* assay needed an additional 1-hour confirmation step when blue colonies were observed on the mFC agar plates. Confirmation is obtained after touching the colony with a micropipette tip or a sterile toothpick, re-suspending it in saline buffer, transferring 1 µL of this suspension to a pre-prepared rtPCR mixture and transferring it to an rtPCR machine for a thermal cycling run of some 50 minutes. In this study, 136 samples from among the 968 well water samples tested resulted in blue colonies on mFC agar; 32 (23.5%) blue colonies isolated from mFC agar were not confirmed as *E. coli* by *E. coli*-specific rtPCR assay. Maheux *et al.* (2011) showed that these false-positive blue colonies are mostly *Acinetobacter baumannii*, *Cronobacter sakazakii*, *E. albertii*, *E. fergusonii*, and *Klebsiella pneumoniae*. This result explains why confirmation tests are required for the mFC

Table 2 | Comparison of methods for detection of *E. coli* presence in well water samples ($n = 968$)

	No. of results by MI		No. of results by mFC + *E. coli*-specific rtPCR	
Method and results	+	−	+	−
MI				
+			79	31
−			33	825
Colilert				
+	78	25	76	29
−	33	832	35	828

Table 3 | Statistical analysis of mFC agar method + *E. coli*-specific rtPCR assay versus MI agar and Colilert culture-based methods for the detection of *E. coli* in well water samples ($n = 968$)

	Colilert				mFC + *E. coli* – specific rtPCR			
Methods	**Index of agreement**	**Cohen kappa**	**McNemar**	***p*-value[a]**	**Index of agreement**	**Cohen kappa**	**McNemar**	***p*-value[a]**
MI	0.94	0.94	1.10	0.2714	0.93	0.93	0.06	0.9522
Colilert					0.93	0.93	0.56	0.5754

[a]A p value <0.05 is necessary to establish a statistically significant difference.

agar method and not for the enzymatic-based methods. Nevertheless, it should be noted that for statistical validity, regions presenting the highest concentrations of *E. coli*-contaminated well water samples were targeted for the sample collection of the present study. Globally, the occurrence of *E. coli* in well water samples ranged mostly from 1% to 5% (Maheux *et al.* 2012). Compared to classical confirmation procedures that require 24 to 48 hours, a confirmation of *E. coli* identity by a 1-h rtPCR could represent a good alternative.

Affordability

In terms of affordability, the MI agar and mFC (+ mEndo) agar methods are comparable. However, the cost of rtPCR confirmation when blue colonies are present makes MI agar more cost-effective since no confirmation is required using this method (Table 4). In all cases, the Colilert method is more expensive (six to 10 times more expensive than MI agar per water sample). However, when no trained employee and/or membrane filtration equipment is available, the Colilert method is more advantageous. For example, in Nunavik, potable water is routinely tested for total coliform and *E. coli* contamination using the Colilert method because it is more user-friendly and less equipment-intensive than standard membrane filtration-based methods (Edberg *et al.* 1989; Martin *et al.* 2007).

Table 4 | Multiparametric comparison of MI agar, Colilert and mFC agar + *E. coli*-specific rt PCR

Parameters	MI	Colilert	mFC + *E. coli*-specific rtPCR
Sensitivity	111/968 (11.5%)	105/968 (10.8%)	111/968 (11.5%)
Easiness	Medium	Easy	Require trained employees
Time-to-result	24 h	24 h	24 h (+ 2 h if presence of blue colonies)
Affordability	1–1.30 USD per sample[a]	6.50–9.80 USD per sample[a]	1.50–1.90 USD per sample[a,b] +2.00 USD/ rtPCR reaction

[a]Cost will vary with the size and with the quote obtained.
[b]This price includes the mEndo agar method for the detection of total coliforms.

CONCLUSIONS

Consequently, using the one-hour *E. coli*-rtPCR assay to confirm the identity of *E. coli* colonies on mFC agar does not provide greater sensitivity than the Colilert and MI agar culture-based methods. Results showed that they are equivalent in term of sensitivity.

Globally, since environmental laboratories already possess the equipment for membrane filtration methods, the use of the MI agar method seems to be the best option for the assessment of drinking water quality by total coliform and *E. coli* detection. MI agar is more cost-effective than Colilert and mFC (+ mEndo) + *E. coli* rtPCR, easier to use and faster than methods requiring confirmation by molecular methods. However, when no trained employee and/or membrane filtration equipment is available, the Colilert method should be preferred.

At the present time, molecular technologies are expensive and not fully automated. However, the day will come when assessing drinking water quality by molecular methods will be more cost-effective and rapid. Furthermore, automation will not require well-trained employees. Then, molecular technologies will compete advantageously with culture-based technologies.

The results obtained in the present study are only applicable to drinking water samples. Results could be different with other types of water.

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