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Comparison of four β-glucuronidase and β-galactosidase-based commercial culture methods used to detect *Escherichia coli* and total coliforms in water

Andrée F. Maheux, Vanessa Dion-Dupont, Sébastien Bouchard, Marc-Antoine Bisson, Michel G. Bergeron and Manuel J. Rodriguez

ABSTRACT

The MI agar, Colilert®, Chromocult coliform® agar, and DC with BCIG agar chromogenic culture-based methods used to assess microbiological quality of drinking water were compared in terms of their ubiquity, sensitivity, ease of use, growth of atypical colonies and affordability. For ubiquity, 129 total coliform (representing 76 species) and 19 *Escherichia coli* strains were tested. Then, 635 1-L well water samples were divided into 100 mL subsamples for testing by all four methods. Test results showed that 70.5, 52.7, 36.4, and 23.3% of the non-*E. coli* total coliform strains and 94.7, 94.7, 89.5, and 89.5% of the 19 *E. coli* strains yielded a positive signal with the four methods, respectively. They also yielded a total coliform positive signal for 66.5, 51.7, 64.9, and 55.0% and an *E. coli* positive signal for 16.1, 14.8, 17.3, and 13.4% of the 635 well water samples tested, respectively. Results showed that Colilert® is the most expensive method tested in terms of reactants, yet it is the easiest to use. Large numbers of atypical colonies were also often observed on Chromocult coliform® and DC with BCIG, thereby challenging the target microorganism count. Thus, the MI agar method seems to be the best option for the assessment of drinking water quality.

Key words | Chromocult coliform® agar, Colilert®, DC with BCIG agar, drinking water, MI agar

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INTRODUCTION

Methods based on the enzymatic properties of coliforms (β-galactosidase for total coliforms and β-glucuronidase enzymes for *Escherichia coli* detection) are used to assess drinking water quality. They were developed to diminish background effects of heterotrophic bacteria and circumvent the need for a confirmation stage required by both multiple-tube fermentation and membrane filter techniques (Clark 1980; Evans *et al.* 1981; Means & Olson 1981; Seidler *et al.* 1981; Burlingame *et al.* 1984; APHA 2005). The β-galactosidase enzyme was chosen because conventional coliform monitoring is based on the detection of the presence of β-galactosidase. The β-glucuronidase enzyme was also chosen

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because the gene encoding this enzyme (*uidA*) was found to be specific (Brenner *et al.* 1972) and present in more than 97% of *E. coli* isolates (Lupo & Halpern 1970; Martins *et al.* 1993).

The MI agar (MI; BD, Franklin Lakes, NJ, USA), Colilert® (IDEXX Laboratories, Westbrook, ME, USA), Chromocult Coliform® agar (Chromocult coliform®; Merk KGaA, Darmstadt, Germany) are three commercial test methods based on the determination of β-galactosidase and β-glucuronidase enzyme activities used to detect, within 24 h, total coliforms and *E. coli* in water samples. These three tests are easy to use, require no additional confirmatory step and provide a more rapid estimate of indicators of the bacteriological contamination of water compared to classical techniques (Brenner *et al.* 1993, 1996; Edberg *et al.* 1988; Horman & Hanninen 2006; Olstadt *et al.* 2007; Pitkanen *et al.* 2007; Hallas *et al.* 2008; Mavridou *et al.* 2010; Boubetra *et al.* 2011). Different collections of strains were tested with each commercial β-galactosidase and β-glucuronisade-based test method to establish their ability to recover total coliforms and *E. coli* strains. All of these methods were found to be at least as efficient as classical reference methods in terms of specificity and sensitivity (Landre *et al.* 1998; Rice *et al.* 1990, 1991, 1993). However, the expression of the β-glucuronidase enzyme was found to be variable depending on the medium and technique used (Chang *et al.* 1989; Shadix & Rice 1991; Feng & Lampel 1994; Maheux *et al.* 2008).

Alternatively, DC with BCIG agar (DC + BCIG; Noegen corporation, Lansing, MI, USA) is formulated to differentiate *E. coli* from other coliforms. Similar to MI, Chromocult coliform®, and Colilert®, the DC + BCIG agar medium contains a chromogenic agent to detect β-glucuronidase enzyme activity. However, it does not contain a chromogenic agent to detect the β-galactosidase enzyme activity. Feng & Hartman (1982) showed that *E. coli* colonies could be distinguished from other coliforms on membrane filters and plates of violet red bile agar if MUG (4-methylumbelliferyl-beta-D-glucuronide) was incorporated into the culture media. According to this, total coliform colonies are pink on DC + BCIG agar with the exception of *E. coli*. Because of the low cost of this medium, DC + BCIG agar could be advantageous to assess drinking water quality. Unfortunately, the performance of DC + BCIG agar as compared to reference methods is not well documented.

In the Province of Québec, the *Programme d'accréditation des laboratoires d'analyse* ('Accreditation program of analytic laboratories'; PALA), is administered by the *Centre d'expertise en analyse environnementale du Québec* (CEAEQ), which certifies private, municipal and institutional laboratories. In 2010, the CEAEQ proposed amending their guidelines and since 2013 requires the measurement of the presence of *E. coli* rather than thermotolerant coliforms, as recommended in the United States and many European countries (AWWA 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. Plenty of methods, including MI, Chromocult coliform®, Colilert®, and DC + BCIG agar, are available to detect the presence of *E. coli* in water with high variability in cost. Currently, there is no study comparing these four methods using both pure cultures of bacteria and water samples.

In this study, we first used a collection of fecal and environmental bacteria isolated from different geographical origins to compare four commercial chromogenic test methods (MI, Colilert®, Chromocult coliform®, and DC + BCIG agar). To our knowledge, this is the first report on the comparison of these test methods, using a pure culture panel of this size. Secondly, we compared the four methods in terms of sensitivity using residential well water samples. Their ability to limit the growth of atypical colonies, ease of use and affordability were also compared. The results of this study will help analytical laboratories to choose the best method according to their own needs.

MATERIALS AND METHODS

Analytical comparison

Bacterial strains

The ability of the four culture-based methods to detect non-*E. coli* total coliforms and *E. coli* strains was verified by using 129 total coliform (representing 76 species) and 19 *E. coli* strains of fecal and environmental origin (Tables 1 and 2). Species identification was reconfirmed using an

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Table 1 | Ability of MI agar, Colilert®, Chromocult coliform® agar, and DC agar with BCIG culture-based methods to detect non-*E. coli* total coliforms strains

		Test methods			
Strains (origin; $n = 129$)	**No. Reference**	**MI agar**	**Colilert®**	**Chromocult coliform® agar**	**DC agar with BCIG**
Budvicia aquatica (environmental)	ATCC 35567	–	Transparent	–	–
Buttiauxella agretis (environmental)	ATCC 33320	Fluorescent	Yellow	Purple	Pink
Cedeca davisae (clinical)	ATCC 33431	Unfluorescent	Transparent	Transparent	Transparent
Cedeca lapagei (clinical)	ATCC 33432	Fluorescent	Pale yellow	Grey	Transparent
Cedeca neteri (clinical)	ATCC 33855	Fluorescent	Pale yellow	Grey	Pink
Citrobacter amalonaticus (clinical)	ATCC 25405	Fluorescent	Pale yellow	Transparent	Transparent
Citrobacter braakii (clinical)	ATCC 43162	Fluorescent	Yellow	Purple	Pink
Citrobacter farmeri (clinical)	ATCC 51112	Fluorescent	Pale yellow	Transparent	Transparent
Citrobacter freundii (food)	ATCC 6879	Fluorescent	Yellow	Purple	Pink
Citrobacter freundii (not available)	ATCC 8454	Fluorescent	Yellow	Purple	Pink
Citrobacter freundii (clinical)	ATCC 8090	Fluorescent	Yellow	Purple	Pink
Citrobacter freundii (environmental)	CCRI-14799	Fluorescent	Yellow	Purple	Pink
Citrobacter freundii (environmental)	CCRI-14827	Fluorescent	Pale yellow	–	–
Citrobacter freundii (environmental)	CCRI-14856	Fluorescent	Yellow	Purple	Pink
Citrobacter gillenii (clinical)	ATCC 51117	Fluorescent	Yellow	Purple	Pink
Citrobacter koseri (clinical)	ATCC 27028	Fluorescent	Pale yellow	Grey	Transparent
Citrobacter koseri (clinical)	ATCC 27156	Fluorescent	Yellow	Purple	Transparent
Citrobacter koseri (clinical)	ATCC 29225	Fluorescent	Yellow	Purple	Transparent
Citrobacter murliniae (clinical)	ATCC 51641	Fluorescent	Yellow	Purple	Transparent
Citrobacter sedlakii (clinical)	ATCC 51115	Fluorescent	Yellow	Grey	Transparent
Citrobacter sedlakii (clinical)	ATCC 51493	Fluorescent	Yellow	–	–
Citrobacter werkmanii (clinical)	ATCC 51114	Fluorescent	Yellow	Purple	Transparent
Citrobacter youngae (food)	ATCC 29935	Fluorescent	Yellow	Purple	Transparent
Cronobacter muytjensii (not available)	ATCC 51329	Fluorescent	Yellow	Purple	Transparent
Cronobacter sakazakii (not available)	ATCC 29004	Unfluorescent	Yellow	Purple	Yellow
Cronobacter sakazakii (clinical)	ATCC 29544	Unfluorescent	Yellow	Purple	Pink
Cronobacter sakazakii (environmental)	CCRI-17037	Fluorescent	Yellow	Purple	Beige
Enterobacter aerogenes (clinical)	ATCC 13048	Fluorescent	Yellow	Purple	Pink
Enterobacter aerogenes (not available)	ATCC 35029	Fluorescent	Yellow	Purple	Pink
Enterobacter aerogenes (not available)	ATCC 51342	Fluorescent	Yellow	Purple	Pink
Enterobacter amnigenus (environmental)	ATCC 33072	Fluorescent	Pale yellow	Purple	Transparent
Enterobacter asburiae (clinical)	ATCC 35954	Fluorescent	Yellow	Purple	Pink
Enterobacter asburiae (clinical)	ATCC 35956	Fluorescent	Yellow	Purple	Pink
Enterobacter cancerogenus (clinical)	ATCC 33241	Fluorescent	Yellow	Purple	Transparent
Enterobacter cancerogenus (clinical)	ATCC 35317	Fluorescent	Yellow	Purple	Transparent
Enterobacter cancerogenus (environmental)	ATCC 49817	Fluorescent	Yellow	Purple	Transparent
Enterobacter cloacae subsp. *cloacae* (clinical)	ATCC 13047	Fluorescent	Yellow	Purple	Transparent
Enterobacter cloacae subsp. *cloacae* (clinical)	ATCC 23355	Fluorescent	Yellow	Purple	Transparent

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Table 1 | continued

Strains (origin; $n = 129$)	No. Reference	Test methods			
		MI agar	Colilert®	Chromocult coliform® agar	DC agar with BCIG
Enterobacter cloacae subsp. *cloacae* (clinical)	ATCC 35588	Fluorescent	Yellow	Purple	Transparent
Enterobacter cloacae subsp. *cloacae* (environmental)	CCRI-17108	Fluorescent	Yellow	Purple	Transparent
Enterobacter cloacae subsp. *dissolvens* (food)	ATCC 23373	Fluorescent	Yellow	Purple	Transparent
Enterobacter gergoviae (clinical)	ATCC 33028	Fluorescent	Pale yellow	Purple	Transparent
Enterobacter gergoviae (clinical)	ATCC 33426	Fluorescent	Pale yellow	Purple	Transparent
Enterobacter gergoviae (clinical)	ATCC 33428	Fluorescent	Pale yellow	Purple	Transparent
Enterobacter hormaechei (clinical)	ATCC 49162	–	Yellow	Purple	Transparent
Enterobacter hormaechei (clinical)	ATCC 49163	Fluorescent	Pale yellow	Grey	Transparent
Enterobacter pyrinus (environmental)	ATCC 49851	Fluorescent	Pale yellow	Pale pink	Transparent
Erwinia amylovora (not available)	ATCC 14976	–	Transparent	–	–
Escherichia blattae (environmental)	ATCC 29907	Unfluorescent	Transparent	Transparent	Transparent
Escherichia fergusonii (clinical)	ATCC 35469	Fluorescent	Pale yellow	Grey	Transparent
Escherichia hermannii (clinical)	ATCC 33650	–	Pale yellow	Grey	Transparent
Escherchia vulneris (food)	ATCC 29943	Fluorescent	Yellow	Purple	Pale pink
Escherichia vulneris (clinical)	ATCC 33821	Fluorescent	Pale yellow	Pale pink	Transparent
Escherichia vulneris (clinical)	ATCC 33832	Fluorescent	Yellow	Purple	Transparent
Ewingella americana (clinical)	ATCC 33852	Unfluorescent	Transparent	Transparent	White
Ewingella americana (clinical)	ATCC 33854	Unfluorescent	Transparent	Transparent	White
Hafnia alvei (clinical)	ATCC 13337	–	Transparent	–	–
Hafnia alvei (not available)	ATCC 25927	Unfluorescent	Transparent	Beige	Transparent
Hafnia alvei (clinical)	ATCC 51873	Fluorescent	Pale yellow	Grey	Transparent
Hafnia alvei (environmental)	CCRI-16651	Unfluorescent	Transparent	Transparent	Transparent
Klebsiella oxytoca (clinical)	ATCC 13182	Fluorescent	Yellow	Grey	Pink
Klebsiella oxytoca (clinical)	ATCC 33496	Fluorescent	Yellow	Grey	White
Klebsiella oxytoca (clinical)	ATCC 41931	Fluorescent	Yellow	Purple	Pale pink
Klebsiella pneumoniae (clinical)	ATCC 27736	Fluorescent	Yellow	Purple	Pink
Klebsiella pneumoniae (environmental)	CCRI-17014	Fluorescent	Yellow	Blue	Blue
Klebsiella pneumoniae (environmental)	CCRI-17064	–	Transparent	–	–
Klebsiella pneumoniae (environmental)	CCRI-17074	Fluorescent	Yellow	Grey	Transparent
Kluyvera ascorbata (clinical)	ATCC 33433	Fluorescent	Yellow	Purple	Pink
Kluyvera ascorbata (not available)	ATCC 33434	Unfluorescent	Yellow	Purple	Pink
Kluyvera cryocrescens (environmental)	ATCC 14239	Fluorescent	Yellow	Purple	Pink
Kluyvera cryocrescens (clinical)	ATCC 33435	Unfluorescent	Yellow	Purple	Pink
Kluyvera georgiana (clinical)	ATCC 51603	Fluorescent	Yellow	Purple	Pink
Kluyvera georgiana (clinical)	ATCC 51702	Fluorescent	Yellow	Purple	Beige
Kluyvera intermedia (environmental)	ATCC 33110	Fluorescent	Transparent	Grey	Pink
Leclercia adecarboxylata (environmental)	ATCC 23216	Fluorescent	Yellow	Pink	Pink

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Table 1 | continued

Strains (origin; $n = 129$)	No. Reference	Test methods			
		MI agar	Colilert®	Chromocult coliform® agar	DC agar with BCIG
Leclercia adecarboxylata (clinical)	ATCC 27984	Fluorescent	Yellow	Pink	Transparent
Moellerella wisconsensis (clinical)	ATCC 35017	Fluorescent	Yellow	Purple	Pink
Pantoea agglomerans (clinical)	ATCC 27155	Unfluorescent	Transparent	Transparent	Transparent
Pantoea dispersa (environmental)	ATCC 14589	Unfluorescent	Transparent	Transparent	Transparent
Providencia rettgeri (not available)	ATCC 29944	Unfluorescent	Transparent	Beige	White
Rahnella aquatilis (environmental)	ATCC 33071	Fluorescent	Yellow	Grey	Pink
Raoutella ornithinolytica (clinical)	ATCC 31898	Fluorescent	Yellow	Grey	White
Raoutella planticola (environmental)	ATCC 33531	Fluorescent	Yellow	Purple	Pink
Raoutella terrigena (environmental)	ATCC 33257	Fluorescent	Pale yellow	Blue	White
Salmonella bongori (not available)	ATCC 43975	Fluorescent	Yellow	Grey	Transparent
Salmonella enterica subsp. *enterica* (clinical)	ATCC 14028	Unfluorescent	Yellow	Transparent	Transparent
Salmonella enterica subsp. *houtenae* (clinical)	ATCC 43974	Unfluorescent	Transparent	Pale yellow	Transparent
Salmonella enterica subsp. *salamae* (clinical)	ATCC 43972	Unfluorescent	Transparent	Transparent	Transparent
Serratia entomophila (environmental)	ATCC 43705	Fluorescent	Pale yellow	Pale yellow	Transparent
Serratia ficaria (environmental)	ATCC 33105	Fluorescent	Pale yellow	Transparent	White
Serratia fonticola (environmental)	ATCC 29844	Fluorescent	Transparent	Grey	Pink
Serratia grimesii (not available)	ATCC 14460	Fluorescent	Yellow	Transparent	Transparent
Serratia liquefaciens (food)	ATCC 27592	Fluorescent	Yellow	Transparent	Transparent
Serratia liquefaciens (food)	ATCC 25641	Fluorescent	Pale yellow	Pale yellow	Transparent
Serratia marcescens (not available)	ATCC 8100	Fluorescent	Pale yellow	Transparent	Transparent
Serratia marcescens (clinical)	ATCC 29021	Fluorescent	Yellow	Transparent	Beige
Serratia marcescens (not available)	ATCC 43862	Fluorescent	Yellow	Pink	Pale pink
Serratia odorifera (clinical)	ATCC 33077	Fluorescent	Yellow	Grey	White
Serratia odorifera (clinical)	ATCC 33132	Fluorescent	Yellow	Grey	Beige
Serratia odorifera (clinical)	ATCC 33133	Fluorescent	Yellow	Pale pink	Transparent
Serratia plymuthica (environmental)	ATCC 183	Fluorescent	Yellow	Grey	White
Serratia proteamaculans subsp. *quinovora* (food)	ATCC 33765	Fluorescent	Yellow	Grey	White
Serratia rubidaea (not available)	ATCC 27593	Fluorescent	Yellow	Grey	Pink
Serratia rubidaea (clinical)	ATCC 29023	Fluorescent	Yellow	Grey	Pink
Shigella boydii (clinical)	ATCC 9207	–	Transparent	Grey	Blue
Shigella dysenteriae (clinical)	ATCC 11835	Unfluorescent	Transparent	Transparent	Transparent
Shigella flexneri (clinical)	ATCC 12022	Unfluorescent	Transparent	Transparent	Transparent
Trabulsiella guamensis (environmental)	ATCC 49490	Fluorescent	Yellow	Transparent	Transparent
Vibrio gazogenes (environmental)	ATCC 43939	Unfluorescent	Transparent	Transparent	Transparent
Yersinia aldovae (environmental)	ATCC 35236	Unfluorescent	Transparent	Transparent	Transparent
Yersinia aldovae (food)	ATCC 35237	–	Transparent	–	–
Yersinia bercovieri (environmental)	ATCC 43970	Fluorescent	Transparent	Transparent	Transparent

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Table 1 | continued

Strains (origin; $n = 129$)	No. Reference	Test methods			
		MI agar	Colilert®	Chromocult coliform® agar	DC agar with BCIG
Yersinia enterocolitica subsp. *enterocolitica* (clinical)	ATCC 9610	–	Transparent	Transparent	Transparent
Yersinia frederiksenii (clinical)	ATCC 29912	Fluorescent	Pale yellow	Transparent	White
Yersinia frederiksenii (environmental)	ATCC 33641	Fluorescent	Yellow	Transparent	Transparent
Yersinia intermedia (clinical)	ATCC 29909	Unfluorescent	Pale yellow	–	–
Yersinia intermedia (clinical)	ATCC 33647	Fluorescent	Pale yellow	Transparent	Transparent
Yersinia intermedia (clinical)	ATCC 33648	Fluorescent	Pale yellow	Transparent	Transparent
Yersinia kristensenii (clinical)	ATCC 33638	–	Transparent	Transparent	–
Yersinia pseudotuberculosis (animal)	ATCC 13979	Unfluorescent	Transparent	Transparent	–
Yersinia pseudotuberculosis (animal)	ATCC 27802	Unfluorescent	Transparent	Transparent	Transparent
Yersinia pseudotuberculosis (animal)	ATCC 29833	–	Transparent	Transparent	Transparent
Yersinia rohdei (animal)	ATCC 43380	Unfluorescent	Transparent	Transparent	–
Yersinia rohdei (animal)	ATCC 43871	Unfluorescent	Transparent	Transparent	–
Yersinia rohdei (clinical)	ATCC 43873	Fluorescent	Pale yellow	Transparent	–
Yersinia ruckeri (animal)	ATCC 29473	Unfluorescent	Transparent	Transparent	Transparent
Yokenella regenburgei (clinical)	ATCC 35313	Fluorescent	Transparent	Beige	Transparent
Yokenella regenburgei (clinical)	ATCC 43001	Unfluorescent	Transparent	Transparent	Transparent
Yokenella regenburgei (clinical)	ATCC 43003	Fluorescent	Pale yellow	Transparent	Transparent
	Total positives:	91/129 (70.5%)	68/129 (52.7%)	47/129 (36.4%)	30/129 (23.3%)

Shading = Positive results.
'–': no growth.
CCRI: Centre de recherche en infectiologie strain collection.

automated MicroScan Autoscan-4 system (Siemens Healthcare Diagnostic Inc., Newark, DE, USA) or a Vitek 32 system (bioMérieux SA, Marcy l'Étoile, France). Bacterial strains were grown from frozen stocks kept at −80 °C in Brucella medium (Beckton, Dickinson and Company, Mississauga, Ontario, Canada) containing 10% glycerol, and cultured on brain-heart infusion (BHI) agar. Three passages were performed prior to analysis of each strain with each culture-based method.

Culture-based methods

Preparation of the bacterial cell suspension

Non-*E. coli* total coliform and *E. coli* cells were grown to the logarithmic phase (0.5–0.6 optical density measured at 600 nm (OD_{600})) in BHI broth and adjusted to a 0.5 McFarland standard (Fisher Scientific Company, Ottawa, Ontario, Canada), before being serially diluted ten-fold in phosphate-buffered saline (PBS; 137 mM NaCl, 6.4 mM Na_2HPO_4, 2.7 mM KCl, 0.88 mM KH_2PO_4, pH 7.4). An aliquot of the 10^{-5} dilution was spiked in sterile reverse osmosis-purified water (resistivity of 18 MΩ·cm min at 25 °C) to produce suspensions containing approximately 50 colony-forming units (CFU) per 100 mL of water. Bacterial counts were verified by filtering 100 mL of each spiked water sample through a Millipore membrane filter (47 mm diameter, 0.45 μm pore size; Millipore Corporation, Billerica, MA, USA) with a standard platform manifold (Millipore Corporation) followed by incubation on BHI agar for 24 ± 2 h at 35.0 ± 0.5 °C. Tests to confirm the sterility of filter membranes and buffer used for rinsing the filtration apparatus were also performed.

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Table 2 | Ability of MI agar, Colilert®, Chromocult coliform® agar, and DC agar with BCIG culture-based methods to detect *E. coli* strains

		Test methods			
Strains (origin; $n = 19$)	**No. Reference**	**MI agar**	**Colilert®**	**Chromocult coliform® agar**	**DC agar with BCIG**
Escherichia coli (clinical)	ATCC 11775	Fluorescent	Yellow	Blue	Blue
Escherichia coli (clinical)	ATCC 23511	Fluorescent	Yellow	Blue	Blue
Escherichia coli (clinical)	ATCC 35401	Fluorescent	Yellow	Blue	Blue
Escherichia coli (clinical)	ATCC 43886	Fluorescent	Yellow	Blue/purple	Pink
Escherichia coli (clinical)	ATCC 43890	Fluorescent	Yellow	Transparent	Transparent
Escherichia coli (clinical)	ATCC 43894	Fluorescent	Yellow	Purple	Pink
Escherichia coli (clinical)	ATCC 43895	Fluorescent	Yellow	Purple	Pink
Escherichia coli (clinical)	ATCC 43896	Fluorescent	Yellow	Blue/purple	Blue
Escherichia coli (clinical)	LSPQ 2086	Fluorescent	Yellow	Blue/purple	Blue
Escherichia coli (clinical)	LSPQ 2092	Fluorescent	Yellow	Blue	Purple
Escherichia coli (clinical)	LSPQ 2113	Fluorescent	Transparent	Grey	–
Escherichia coli (clinical)	LSPQ 2115	Fluorescent	Yellow	Blue	Blue
Escherichia coli (clinical)	LSPQ 2117	–	Yellow	Blue	Blue
Escherichia coli (clinical)	LSPQ 2118	Fluorescent	Yellow	Blue/purple	Pink
Escherichia coli (clinical)	LSPQ 2125	Fluorescent	Yellow	Blue/purple	Pink
Escherichia coli (clinical)	LSPQ 2127	Fluorescent	Yellow	Blue/purple	Pink
Escherichia coli (clinical)	LSPQ 3760	Fluorescent	Yellow	Blue/purple	Pink
Escherichia coli (clinical)	LSPQ 3761	Fluorescent	Yellow	Blue/purple	Pink
Escherichia coli (clinical)	LSPQ 3762	Fluorescent	Yellow	Blue/purple	Pink
	Total positives:	18/19 (94.7%)	18/19 (94.7%)	17/19 (89.5%)	17/19 (89.5%)

Shading = Positive results.
'–': no growth.

Membrane filtration method

The membrane filtration method was performed according to Maheux *et al.* (2009). Three 100 mL volumes were filtered on Millipore filters with a standard platform manifold. The first filter was incubated on MI agar (MI; BD, Franklin Lakes, NJ, USA), the second filter was incubated on Chromocult coliform® agar (Chromocult coliform®, Merk KGaA, Darmstadt, Germany), and the third filter was incubated on DC with BCIG agar (DC + BCIG; Neogen Corporation, Lansing, MI, USA) for 24 ± 2 h at $35.0 \pm 0.5\,^{\circ}$C, before determining colony counts and colour. Each preparation of MI, Chromocult coliform®, and DC + BCIG plates was tested for performance using positive and negative control strains (*Enterobacter aerogenes* ATCC 13048, *E. coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853), as recommended by the manufacturer's labeled instructions and the USEPA microbiology methods manual. Tests to confirm the sterility of the filter membranes and buffer used for rinsing the filtration apparatus were also performed (APHA 2005).

Liquid culture method

For the detection of total coliform and *E. coli* strains with Colilert® (Colilert®; IDEXX Laboratories Canada Corp., Toronto, Ontario, Canada), all preparation, validation, storage and handling steps were performed according to the manufacturer's instructions. Briefly, one snap pack containing the Colilert® reagent was dissolved in 100 mL of spiked water samples. The solution was then added to a Quanti-tray®, sealed and incubated at $35.0 \pm 0.5\,^{\circ}$C for 24 ± 2 h prior to the identification of total coliform positive samples presenting yellow colouration and *E. coli* samples presenting both yellow colouration and fluorescence under UV light ($\lambda = 365$ nm).

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Comparison using well water samples

Sample collection

During the summer of 2012, 635 1-L raw well water samples from individual households were collected in the Québec City region (Canada). Each well water sample was divided into 100 mL subsamples for simultaneous testing by standard microbiological methods using MI, Chromocult coliform®, DC + BCIG, and Colilert® (see the 'Membrane filtration method' and 'Liquid culture method' sections).

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).

To determine the ubiquity (ability to detect all or most total coliform strains) species identification by MicroScan Autoscan-4 system or the Vitek 32 system was used as a reference. Ubiquity was calculated by dividing the number of strains detected by the test by the number of total coliform strains tested.

All water samples were recorded as positive (1) or negative (0) for total coliforms and *E. coli*. No method was used as a reference to determine the specificity and sensitivity of a particular test for the detection of total coliforms and *E. coli*. All the methods were compared to each other. Sensitivity (true positive rate) was calculated by dividing the number of positive samples by Method No. 1 plus positive samples by Method No. 2 by the number of positive samples in Method No. 2. Specificity (true negative rate) was calculated by dividing the number of negative samples by Method No. 1 plus negative samples by Method No. 2 by the number of negative samples in Method No. 2.

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 is a significant difference between both methods.

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, 0.40–0.75 = fair to good, and <0.40 = poor).

RESULTS

Analytical detection of total coliform strains

One hundred and twenty-nine total coliform strains (representing 76 species) from fecal and environmental settings were used to demonstrate the ability of MI agar (MI), Colilert®, Chromocult coliform® agar (Chromocult coliform®), and DC with BCIG agar (DC + BCIG) culture-based methods to detect various total coliform strains (ubiquity; Table 1). The results obtained showed that 91 (70.5%), 68 (52.7%), 47 (36.4%) and 30 (23.3%) of the 129 non-*E. coli* total coliform strains tested yielded a positive signal with the MI, Colilert®, Chromocult coliform®, and DC + BCIG methods, respectively. No relationship was observed between isolate origin and false-negative results.

Analytical detection of *Escherichia coli* strains

Nineteen *E. coli* strains from fecal and environmental settings as well as from different geographic origins were used to demonstrate the ability of the four culture methods to detect various *E. coli* strains (ubiquity; Table 2). For confirmation purposes, all strains that presented negative results were also tested a second time with a different lot of kit/media. The results obtained showed that 18 (94.7%), 18 (94.7%), 17 (89.5%) and 17 (89.5%) of the 19 *E. coli* strains tested yielded a positive signal with MI, Colilert®, Chromocult coliform®, and DC + BCIG methods, respectively.

Ability of MI, Colilert®, Chromocult coliform®, and DC + BCIG agar to detect total coliforms and *E. coli* from well water samples

Six hundred and thirty-five 1-L well water samples collected in the Québec City region during the summer of 2012 were divided into 100 mL subsamples for testing by all four methods to verify how these observations are transposed when real well water samples are tested. The MI, Colilert®, Chromocult coliform®, and DC + BCIG culture-based

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Table 3 | Ability of MI agar, Colilert®, Chromocult coliform® agar, and DC agar with BCIG culture-based methods to detect total coliforms and *E. coli* from potable water samples

	MI agar	Colilert®	Chromocult coliform® agar	DC agar with BCIG
	(CFU/100 mL)	(MPN/100 mL)	(CFU/100 mL)	(CFU/100 mL)
Total coliform				
Positive result ([1–9])	155/635 (24.4%)	176/635 (26.3%)	156/635 (24.6%)	163/635 (25.7%)
Positive result (≥10)	267/635 (42.0%)	161/635 (25.4%)	256/635 (40.3%)	186/635 (29.3%)
Total	422/635 (66.5%)	328/635 (51.7%)	412/635 (64.9%)	349/635 (55.0%)
E. coli				
Total	102/635 (16.1%)	94/635 (14.8%)	110/635 (17.3%)	85/635 (13.4%)

methods yielded a total coliform positive signal for 422 (66.5%), 328 (51.7%), 412 (63.9%) and 349 (55.0%) of 635 well water samples tested, respectively, while only 267 (42.0%), 161 (25.4%), 256 (40.3%) and 186 (29.3%) exceeded the concentration of 10 total coliform CFU/100 mL, respectively (Tables 3, 4 and 6). For each method, an *E. coli* positive signal was observed for 102 (16.1%), 94 (14.8%), 110 (17.3%) and 85 (13.4%), respectively, of the 635 well water samples tested (Tables 3, 5 and 7).

Growth of atypical colonies

For the 635 1-L well water samples tested, 85 (13.3%) and 80 (12.6%) allowed the growth of more than 200 atypical colonies on the filter for Chromocult coliform® and DC + BICG agar, respectively, whereas only six (0.9%) filters out of 635 contained more than 200 atypical colonies for the MI method.

Table 4 | Comparison of methods for detection of total coliform presence in well water samples ($n = 635$)

	No. of results by MI agar		No. of results by Colilert®		No. of results by Chromocult coliform® agar	
Method and results	+	−	+	−	+	−
Colilert®						
+	306	23				
−	116	190				
Chromocult coliform® agar						
+	376	36	303	109		
−	46	177	25	198		
DC agar with BCIG						
+	333	20	290	63	324	26
−	90	192	38	244	90	190

DISCUSSION

Analytical detection of total coliform strains

In the present study, the ability of the four culture-based methods tested to detect total coliform strains was statistically different: MI agar (MI) presented the best detection level and DC with BCIG (DC + BCIG) agar the worst, with a difference of 47.2% between the two methods. Detection of total coliforms on the DC + BCIG method is not obvious. Indeed, contrary to the three other methods tested, the medium does not contain a chromogenic agent for β-galactosidase detection. It contains only a chromogenic agent for β-glucuronidase detection. Thus, on this medium, pink colonies are considered total coliforms. Therefore, contrary to the three other methods tested, identification tests of typical colonies should be conducted to confirm the results obtained. Similar to Maheux *et al.* (2008), the results of the present study lacked correlation between test methods based on the same enzymatic principle to recognize a strain as non-*E. coli* total coliform. Indeed, our results showed that there is no correlation between the four methods tested either within the same genera or the same species (Table 1).

In 2008, Maheux tested 33 reference and environmental non-*E. coli* total coliform strains (representing 26 species) to demonstrate the ability of MI, Colilert®, Chromocult coliform® agar (Chromocult coliform®) and Readycult®

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Table 5 | Comparison of methods for detection of *E. coli* presence in well water samples ($n = 635$)

	No. of results by MI agar		No. of results by Colilert®		No. of results by Chromocult coliform® agar	
Method and results	+	−	+	−	+	−
Colilert®						
+	74	20				
−	28	513				
Chromocult coliform® agar						
+	81	29	68	42		
−	21	504	23	502		
DC agar with BCIG						
+	75	13	63	24	71	17
−	27	520	32	516	39	508

culture-based methods to detect various total coliform strains. They showed that the β-galactosidase of 15 (45.5%), 20 (60.6%), 19 (57.6%), and 19 (57.6%) of the total coliform strains tested was detected by the four methods, respectively. For confirmation purposes in this study, all strains that had presented negative results during testing by Maheux *et al.* (2008) were tested a second time with a different lot of kit/media. However, among the β-galactosidase-negative strains tested by Maheux *et al.* (2008; ATCC 43890, ATCC 43894, ATCC 43895, LSPQ 2127, LSPQ 3760, LSPQ 3761, and LSPQ 3762) on MI agar, β-galactosidase production was detected during this study. This observation seems to confirm the assumption suggesting that identification methods relying solely on the activity of a single enzyme are subject to a lack of robustness and may lead to misinterpretations since enzymatic activity can be transient and highly regulated by environmental factors (Maheux *et al.* 2008).

Analytical detection of *E. coli* strains

Based on the results obtained, the four culture-based methods tested are not statistically different using pure *E. coli* cultures. However, it should be noted that for each *E. coli* strain tested, β-glucuronidase production was detected with at least one of the four methods. Once again, this observation seems to confirm that enzymatic activity can be transient and regulated by environmental factors, including the composition of culture media.

Ability of MI, Colilert®, Chromocult coliform®, and DC + BCIG agar to detect total coliforms and *E. coli* from well water samples

The MI method detected significantly more total coliform-positive well water samples than Colilert® and DC + BCIG agar (Table 6). For the detection of *E. coli*-positive water samples, all enzymatic culture-based methods tested were equivalent with the exception of DC + BCIG agar that detected statistically fewer *E. coli*-positive well water samples than the other three methods.

Growth of atypical colonies

The MI method is more specific than the Chromocult coliform® and DC + BCIG agar methods since fewer atypical colonies grew on MI compared to the other two methods.

Table 6 | Statistical analysis of the four chromogenic culture-based methods for the detection of total coliform in well water samples ($n = 635$)

	MI agar				Colilert®				Chromocult coliform® agar			
Methods	**Index of agreement**	**Cohen kappa**	**McNemar**	***p* value[a]**	**Index of agreement**	**Cohen kappa**	**McNemar**	***p* value[a]**	**Index of agreement**	**Cohen kappa**	**McNemar**	***p* value[a]**
Colilert®	0.78	0.72	62.22	<0.0001								
Chromocult coliform® agar	0.87	0.85	1.22	0.2224	0.79	0.73	52.66	<0.0001				
DC agar with BCIG	0.83	0.79	44.55	<0.0001	0.84	0.81	6.19	<0.0001	0.82	0.78	35.31	<0.0001

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

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Table 7 | Statistical analysis of the four chromogenic culture-based methods for the detection of *E. coli* in well water samples ($n = 635$)

	MI agar				Colilert®				Chromocult coliform® agar			
Methods	Index of agreement	Cohen kappa	McNemar	*p* value[a]	Index of agreement	Cohen kappa	McNemar	*p* value[a]	Index of agreement	Cohen kappa	McNemar	*p* value[a]
Colilert®	0.92	0.92	1.33	0.1836								
Chromocult coliform® agar	0.92	0.91	1.28	0.2006	0.90	0.89	5.55	<0.0001				
DC agar with BCIG	0.94	0.93	4.90	<0.0001	0.91	0.90	1.14	0.2542	0.91	0.90	8.64	<0.0001

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

As a liquid culture method, the growth of atypical colonies could not be investigated for the Colilert® culture-based method.

Time to result

In terms of time to result, all four methods tested comparably since they required 24 hours for results. However, contrary to MI, Colilert, and Chromocult coliform® methods, suspect total coliform colonies on DC + BCIG agar should be confirmed with additional tests.

Ease of use

In terms of ease of use, the Colilert® method was the easiest to use. The unit-dosed packaging eliminates media preparation. Furthermore, there is no repeat testing due to clogged filters. Finally, contrary to other membrane filtration-based methods, its use does not require well-trained employees. The MI, Chromocult coliform®, and DC + BCIG agar methods provided comparable ease of use in terms of membrane filtration methods. Media must also be prepared and quality control carried out for each batch. Employee training is also more important than for the Colilert® method. However, employees already using membrane filtration equipment can easily use these methods (Table 8).

Affordability

In terms of affordability, the Chromocult coliform® and DC + BCIG agar are comparable. MI is approximately 30% more expensive than the two previous methods. Colilert® reactants are more expensive (six to 10 times more expensive than chromogenic membrane filtration-based reactants per water sample; Table 8). However, the cost associated with employees is higher for chromogenic membrane filtration-based methods than for Colilert® since the latter is much easier to use.

In water management, multiple parameters will influence the choice of an analytical method to assess drinking water. Despite the fact that MI, Colilert®, and Chromocult coliform® have been shown equivalent in terms of specificity and sensitivity, the ease of use and the cost will also influence the choice of a method. In this study, we addressed all these parameters to help authorities and analytical laboratories make a choice among all available methods for the purpose of their own needs.

Table 8 | Comparison of MI agar, Colilert®, Chromocult coliform® agar, and DC agar with BCIG enzymatic culture-based methods in terms of ease of use and affordability

Parameters	MI agar	Colilert®	Chromocult coliform® agar	DC agar with BCIG
Ease of use	Medium	Easy	Medium	Medium
Affordability	1–1.30 USD per sample[a]	6.50–9.80 USD per sample[a]	0.75–1.00 USD per sample[a]	0.80–1.05 USD per sample[a]

[a]Cost will vary with the size and with the quote obtained.

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CONCLUSION

We conducted a multiparametric comparison study of the MI agar, Colilert®, Chromocult coliform agar and DC with BCIG agar methods in terms of ubiquity and sensitivity using both pure cultures of bacteria and residential well water samples. We also compared their ability to limit the growth of atypical colonies, ease of use and affordability. To our knowledge, this is the first report on the comparison of these test methods using a pure culture panel of this size. We showed that, 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 even if it costs 30% more than other chromogenic membrane filtration methods. Indeed, MI agar is more cost-effective than Colilert® and more specific than the Chromocult coliform agar and DC with BCIG agar methods which showed more growth of atypical colonies. However, when no trained employee and/or no membrane filtration equipment are available, the Colilert® method should be preferred. The results obtained in the present study are applicable solely to drinking water samples. Results could differ with other types of water.

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