Comparison of two methods for evaluating the quality of stored drinking water in Abidjan, Côte d'Ivoire, and review of other comparisons in the literature

J. T. Macy, E. F. Dunne, Y. H. Angoran-Benie, Y. Kamelan-Tano, L. Kouadio, K. A. Djai and S. P. Luby

ABSTRACT

Membrane filtration, multiple tube fermentation (the standard methods) and Colilert are techniques available for assessing drinking water quality, but there are no published comparisons of Colilert to standard methods in a developing country laboratory. We reviewed the published literature on Colilert and standard methods and conducted a study to compare Colilert with membrane filtration for the detection and enumeration of total coliforms and fecal coliforms (*Escherichia coli* bacteria) using 35 stored drinking water samples from households in Abidjan, Côte d'Ivoire. Our study results are consistent with previous published studies conducted in developed countries. Results from Colilert and membrane filtration correlated for both total coliforms ($r^2 = 0.81$) and *E. coli* ($r^2 = 0.93$). Colilert is an acceptable method to measure the presence and quantity of coliforms in water samples in a developing country setting.

Key words | Colilert, *E. coli*, Ivory Coast, standard methods, total coliforms, water quality

J. T. Macy
E. F. Dunne
S. P. Luby (corresponding author)
Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases,
National Center for Infectious Diseases, Centers for Disease Control and Prevention,
1600 Clifton Road, NE, MS A-38,
Atlanta, GA 30333,
USA
Tel: (404) 639-2206
Fax: (404) 639-2205
E-mail: sxl2@cdc.gov

Y. H. Angoran-Benie
Y. Kamelan-Tano
Projet RETRO-CI,
Abidjan, Côte d'Ivoire

L. Kouadio
K. A. Djai
National Institute of Hygiene,
Abidjan, Côte d'Ivoire

INTRODUCTION

The World Health Organization (WHO) recommends the measurement of *E. coli* in drinking water samples as the best indicator of water quality. The WHO guideline for potable water is less than one *E. coli* per 100 ml of drinking water (World Health Organization 1998). Multiple tube fermentation, membrane filtration and Colilert (IDEXX Laboratories, Inc., Westbrook, Maine) are laboratory methods used to qualify or quantify the level of bacteria in drinking water samples. The multiple tube fermentation and membrane filtration tests measure total coliforms and *E. coli* and are standard methods for water quality assessments. Both tests assess the number of bacteria based on lactose fermentation with production of sheen colonies, gas, or acid and gas. Results from the multiple tube fermentation method estimate the most probable number (MPN) of coliforms or *E. coli* per 100 ml after growth of coliforms in liquid medium. Results from the membrane filtration method approximate the number of coliforms or *E. coli* colonies per 100 ml after growth of bacteria on the surface of agar.

Colilert is a recently available method to determine the MPN of coliforms. Colilert uses defined substrate technology to detect and quantify total coliforms and *E. coli* from water samples (Edberg & Edberg 1988). As coliforms grow, they use β-galactosidase to metabolize the nutrient indicator o-nitrophenyl-β-D-galactopyranoside and change it from colourless to yellow. *E. coli* use β-glucuronidase to metabolize 4-methylumbelliferyl-β-D-glucuronide, which creates a molecule that fluoresces under ultraviolet light. Colilert is simpler to use, allows greater throughput, and requires less time to standardize than standard methods.

*Inclusion of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention or the United States Department of Health and Human Services.

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It detects total coliforms and *E. coli* simultaneously in 24 hours or less and confirmation is not needed. The US Environmental Protection Agency approved Colilert for drinking water monitoring in 1992 (Federal Register 1989; Federal Register 1992), and it is used in many developed country settings.

In developing countries, laboratories commonly use membrane filtration and multiple tube fermentation for water quality assessments. The Colilert method is available, but there are no published comparisons of Colilert with standard methods in a developing country laboratory. The data available from developed countries may not be applicable to a developing country setting because of differences in both water quality and laboratory conditions. Many developing countries are in the tropics, where water is more likely to have a higher level of organic material and a greater variety of organisms (Toranzos 1991; World Health Organization & United Nations Children's Fund 2000). Accurate results from membrane filtration require high levels of technical skill and quality control that are not always available in developing country laboratories. Although Colilert is more costly than membrane filtration, it may be a better option for some laboratories in developing countries because of the ease of use in the laboratory. To evaluate the utility of Colilert for water quality assessments in a developing country setting, we reviewed the published literature on Colilert and standard methods, and we compared Colilert with membrane filtration using samples from a study of stored drinking water in Abidjan, Côte d'Ivoire (Dunne *et al.* 2001).

METHODS

Literature review

We conducted a MEDLINE search using the search term Colilert for the period 1966 to March 2002 for published literature reporting the results of comparisons of Colilert with membrane filtration or multiple tube fermentation. We also included references cited in the articles identified, but not located in the MEDLINE search. We included studies published in English in peer-review journals.

Water samples

We conducted this study from April 1999 to June 1999 in the Koumassi district of Abidjan, consisting mostly of households of lower socio-economic status. To evaluate the water treatment and distribution for municipal water in Abidjan, we visited two water treatment sites, Bingerville and Riviera Nord. Riviera Nord is one of two water treatment sites that provide water to Koumassi. Water is collected at these sites from deep wells, stored in tanks, and piped to consumers through the municipal water system. Free chlorine levels in water leaving these facilities were maintained between 0.2 and 0.5 $mg\,l^{-1}$. Most families collect municipal water and store it in plastic containers for drinking or household use; they often remove the water by dipping a cup in the opening of the storage container. We collected stored drinking water samples from 120 households; in the first 35 households we collected water for comparison of Colilert with membrane filtration methods. We collected water samples in two 300-ml Whirl-paks (Nasco International, Inc., Ft Atkinson, Wisconsin), one of which was impregnated with thiosulfate. We transported the samples with thiosulfate in a cooler with ice to the laboratory for evaluation within 6 hours. We tested the physical characteristics of the samples and evaluated the samples by membrane filtration and Colilert for total coliform bacteria colony counts and *E. coli* colony counts.

Physical characteristics

Water samples without thiosulfate were evaluated for free and total chlorine levels with a Hach digital chlorimeter (Loveland, Colorado). Turbidity was measured with a Hach 2100P portable turbidimeter (Loveland, Colorado), and pH concentration was measured with a Microprocessor pH320 pH meter (WTW, Germany). Nitrate, nitrite and ammonium levels were measured with a Palintest photometer (Gateshead, UK).

Membrane filtration method

Depending on the level of free chlorine in the sample, we made 1 to 3 dilutions. If there was evidence of at least

0.15 mg l^{-1} of free chlorine in a sample, we did not dilute the sample. Otherwise, we made dilutions of 1:10, 1:100 and 1:1,000. We repeated this procedure twice for each sample so that we had duplicates of each sample. We vacuum filtered the samples, placed the filters on m-ColiBlue media (Hach Company, Loveland, Colorado) (Federal Register 1999), and incubated them at 35°C for 24 hours. We counted the total number of colonies of coliform bacteria (red) and *E. coli* (blue) to determine the number of colonies per 100 ml of water. We did not do additional confirmatory testing.

Colilert method

We mixed 100 ml of the water sample with the reagent provided, poured this water into the Colilert tray, and sealed the tray with the Colilert sealer. The Colilert tray consists of 49 large (1.6-ml) and 48 small (120-μl) empty wells that are filled by the water sample and reagent mixture. We placed the sealed Colilert trays in a 35°C incubator. After 24 hours, we counted the number of large and small wells that had changed colour and used the manufacturer's provided table to convert the number of large and small wells to an estimate of the MPN. To determine the MPN of *E. coli*, we counted the number of large and small wells fluorescent with ultraviolet light and referred to the same table. We recorded the MPN for each sample and dilution.

Data analysis

We recorded the results from both methods and entered these data into Epi-Info 6.1 (Centers for Disease Control and Prevention, Atlanta, Georgia) for analysis. For membrane filtration, we determined a result by choosing the dilution with a number of colonies greater than 10 but not too numerous to count (TNTC), defined as greater than 80 colonies. In four samples, we calculated total coliform results using less than 10 colonies because the next lower dilution was TNTC or the next higher dilution yielded no colonies. For *E. coli*, we used 10 samples with less than 10 colonies to determine a result for the same reasons. Because we tested duplicate samples for membrane filtration, we calculated the final result by taking the arithmetic mean of the two results within the same dilution.

To assess agreement between membrane filtration and Colilert on a presence-absence basis, we constructed 2 × 2 tables for coliforms and *E. coli*. To determine a quantitative difference between the membrane filtration and Colilert methods, we calculated the difference in the log of the result. We used a log scale to compare outputs because results were derived from 1:10 dilutions. To compare results with a value of zero, we added one to each result of zero. We reported samples with greater than 80 colonies as TNTC. We excluded these samples from the analysis of the quantitative difference. We used Epi-Info 6.1 to calculate correlation coefficients.

RESULTS

Characteristics of water sampled

We tested the 35 stored water samples for free and total chlorine, turbidity and pH. Free chlorine levels ranged from 0.01 to 0.43 mg l^{-1} (median of 0.04 mg l^{-1}). Total chlorine levels were slightly higher and ranged from 0.01 to 0.47 mg l^{-1} (median of 0.08 mg l^{-1}). Turbidity ranged from 0.37 to 2.85 NTUs (median of 0.97 NTUs), and pH ranged from 6.99 to 7.92 (median of 7.53). Nitrate levels ranged from 0 to 0.71 mg l^{-1} (median of 0.20 mg l^{-1}), nitrite levels ranged from 0 to 0.36 mg l^{-1} (median of 0 mg l^{-1}), and ammonium levels ranged from 0 to 0.6 mg l^{-1} (median of 0.05 mg l^{-1}).

Presence-absence

We compared the results from Colilert and membrane filtration by presence or absence of coliform and *E. coli* bacteria. Of the 35 stored water samples tested for coliforms, 28 (80%) were positive by membrane filtration, and 28 (80%) were positive by Colilert. Twenty-seven samples were positive by both methods, one was positive by membrane filtration but negative by Colilert, one was negative by membrane filtration but positive by Colilert, and six were negative by both methods.

We tested the same 35 stored water samples for *E. coli*. Nineteen (54%) were positive by membrane filtration, and 16 (46%) were positive by Colilert. Sixteen samples were positive by both methods, three were positive by membrane

filtration but negative by Colilert, and 16 were negative by both methods.

Enumeration

We quantified the number of coliform and *E. coli* bacteria in the stored water samples, for multiple dilutions, by both the Colilert and membrane filtration methods. Of the 35 stored water samples tested for coliforms, in 7 (20%) there was no difference between results from Colilert and membrane filtration. Of the remaining 28 samples, 21 (60%) had a less than 1 log difference, 4 (11%) had a greater than 1 but less than 2 log difference, 1 (3%) had a greater than 2 log difference, 1 (3%) had TNTC for both methods, and 1 (3%) had TNTC for Colilert and a high, but countable result for membrane filtration. Figure 1 shows the scatter plot for logarithmic graphs of Colilert compared with membrane filtration results for total coliforms. For 21 (60%) samples, the Colilert measurement of total coliforms was higher than the membrane filtration result, and for 5 (14%) samples, the Colilert measurement was lower. Analysis using simple regression yielded an r value of 0.90 and an r^2 value of 0.81 (95% CI 0.65, 0.90).

Of the 35 stored water samples tested for *E. coli*, there was no difference between results from Colilert and membrane filtration in 18 (51%) samples. The 17 (49%) remaining samples had a less than 1 log difference. Figure 2 shows the scatter plot for logarithmic graphs of Colilert compared with membrane filtration results for *E. coli*.

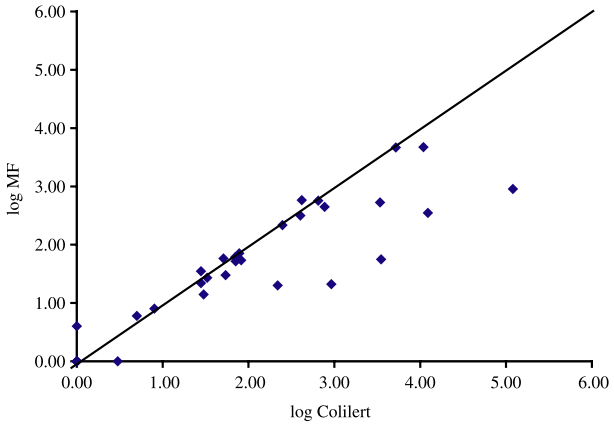

Figure 1 | Scatter plot showing logs of membrane filtration and Colilert results for testing for coliform bacteria in stored water samples, Abidjan, Côte d'Ivoire.

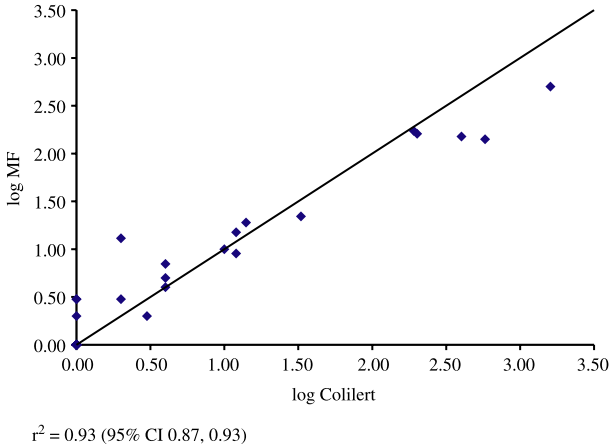

Figure 2 | Scatter plot showing logs of membrane filtration and Colilert results for testing for *E. coli* bacteria in stored water samples, Abidjan, Côte d'Ivoire.

Colilert yielded a higher measurement of *E. coli* concentration for 8 (23%) out of the 35 samples, and a lower result for 9 (26%) samples. Analysis using simple regression yielded an r value of 0.97 and an r^2 value of 0.93 (95% CI 0.87, 0.97).

DISCUSSION

Water quality assessments are an important component of public health activities in developing countries. Using a simpler laboratory method to evaluate water quality might facilitate water quality assessments in rural and underserved areas of developing countries that are not typically evaluated due to inadequate laboratory capacity.

Studies in developed countries have compared Colilert with membrane filtration and with multiple tube fermentation (Table 1). The majority of studies found no statistically significant differences between Colilert and membrane filtration (Edberg *et al.* 1989; Lewis & Mak 1989; Cowburn *et al.* 1994; Fricker *et al.* 1997; Eckner 1998) and Colilert and multiple tube fermentation (Edberg *et al.* 1988, 1989, 1990; Eckner 1998). Other studies, however, reported differences between Colilert and membrane filtration (Olson *et al.* 1991; Schets *et al.* 1993, 2002) and Colilert and multiple tube fermentation (Covert *et al.* 1989; Schets *et al.* 1993; Grasso *et al.* 2000). One study found that Colilert and multiple tube fermentation produced similar results for total coliforms but not for *E. coli* (Gale & Broberg 1993).

Table 1 | Results of studies comparing Colilert with standard methods for detection of total coliforms and *E. coli* in water samples

				Results			
				Total coliforms		*E. coli*	
Study	Location	Methods evaluated	Water sampled	n	Statistical analysis	n	Statistical analysis
Clark *et al.* 1991	US	Colilert and MF	Treated			83	Statistically significant difference (54% agreement; McNemar chi-square test ($P < 0.05$))
			Untreated			32	No statistically significant difference (85% agreement; McNemar chi square test ($P > 0.05$))
Covert *et al.* 1989	US	Colilert and MTF	Untreated	31	Statistically significant difference (Mantel-Haenzel test indicated significant difference in recovery ($P = 0.0004$); no significant difference in precision ($P = 0.6966$))		
Cowburn *et al.* 1994	UK	Colilert and MF	Treated	276	No statistically significant difference	276	No statistically significant difference
			Treated	220	No statistically significant difference	220	No statistically significant difference
			Untreated	129	No statistically significant difference	129	No statistically significant difference
Eckner 1998	Sweden	Colilert and Swedish standard methods (MTF and MF)	Both	247	No statistically significant difference (Spearman rank correlation coefficient = 0.77)	257	No statistically significant difference (Spearman rank correlation coefficient = 0.84)
Edberg *et al.* 1988	US	Colilert and MTF	Treated	46	No statistically significant difference ($r = 0.883$; $r^2 = 0.779$)		
Edberg *et al.* 1989	US	Colilert and standard methods (MTF and MF)	Treated	702	No statistically significant difference (94% agreement; Pearson, Mantel-Haenzel, and McNemar chi-square tests)		
Edberg *et al.* 1990	US	Colilert and MTF	Untreated	47	No statistically significant difference ($r^2 = 0.514$)		

Table 1 | (*continued*)

					Results		
Fricker *et al.* 1997	UK	Colilert and MF	Treated	7389	No statistically significant difference (Correlation coefficient = 0.87)	7389	No statistically significant difference (Correlation coefficient = 0.89)
Gale and Broberg 1993	UK	Colilert and MTF	Untreated	124	No statistically significant difference ($P > 0.5$)	124	Statistically significant difference ($P = 0.001$)
Grasso *et al.* 2000	Italy	Colilert and MTF	Untreated	80	Statistically significant difference ($P < 0.005$)	80	Statistically significant difference ($P < 0.005$)
Lewis & Mak 1989	Canada	Colilert and MF	Treated	950	No statistically significant difference (97% agreement)		
Olson *et al.* 1991	US	Colilert and MF	Both	749	Statistically significant difference (95% agreement; McNemar chi-square = 31.03 ($P < 0.05$))		
Schets *et al.* 1993	Netherlands	Colilert and Dutch Standard Methods	Untreated	12	No statistics reported (10 (83%) samples positive by Colilert, and 12 (100%) positive by Dutch standard methods)	10	No statistics reported (6 (60%) samples positive by Colilert, and 8 (80%) positive by Dutch standard methods)
Schets *et al.* 2002	Netherlands	Colilert and MF	Both	179	Statistically significant difference (Colilert produced higher counts)	179	Statistically significant difference (Colilert produced lower counts and false negative results)

Different conclusions from numerous studies that have compared Colilert with membrane filtration and multiple tube fermentation could result from differences in sampled water. Some water samples, such as surface water, will likely have a greater number and wider variety of microorganisms than other samples. The sources utilized in the studies included well water (Covert *et al.* 1989; Olson *et al.* 1991; Eckner 1998), surface water (Covert *et al.* 1989; Edberg *et al.* 1990; Cowburn *et al.* 1994; Eckner 1998; Grasso *et al.* 2000; Schets *et al.* 2002), water storage reservoirs (Olson *et al.* 1991; Schets *et al.* 1993), water distribution systems (Edberg *et al.* 1988, 1989; Covert *et al.* 1989; Lewis & Mak 1989; Olson *et al.* 1991; Schets *et al.* 2002), disinfected sewage effluent (Cowburn *et al.* 1994; Fricker *et al.* 1997), cisterns (Covert *et al.* 1989), rivers (Gale & Broberg 1993; Schets *et al.* 1993), lakes (Schets *et al.* 1993) and natural springs (Covert *et al.* 1989). Clark *et al.* reported that Colilert and membrane filtration were comparable for detecting *E. coli* in untreated surface water samples, but that the two methods did not perform similarly in the detection of *E. coli* in treated water samples (1991). However, other studies using treated water (Edberg *et al.* 1988, 1989; Lewis & Mak 1989; Cowburn *et al.* 1994; Fricker *et al.* 1997) found that Colilert and standard methods were comparable, whereas studies using untreated water (Covert *et al.* 1989; Gale & Broberg 1993; Schets *et al.* 1993; Grasso *et al.* 2000) found differences between Colilert and standard methods.

In our study, the Colilert method produced similar results to membrane filtration for the presence-absence detection of both coliform and *E. coli* bacteria in stored drinking water samples from a developing country. This is consistent with studies conducted in the United Kingdom (Cowburn *et al.* 1994) and Sweden (Eckner 1998) in which Colilert gave similar results to membrane filtration for the detection of both coliforms and *E. coli*. Our study also demonstrated that Colilert was comparable to membrane filtration for quantitative results for both total coliform and *E. coli* bacteria. Fricker *et al.* (1997) concluded that Colilert was a suitable alternative to membrane filtration for enumeration of total coliforms and *E. coli*. It is important to note that three samples that were positive for *E. coli* by membrane filtration were negative by Colilert. In all of these samples, there were less than 3 colonies per 100 ml detected by membrane filtration.

CONCLUSIONS

Our study demonstrates that Colilert is an acceptable method to measure the presence and quantity of coliform and *E. coli* bacteria in water samples in a developing country setting. These data from one developing country setting support the literature from other settings that Colilert is a reasonable alternative to membrane filtration. Importantly, this is a small study from one developing country site with unusually thoroughly treated water. It would be useful to repeat this assessment using water with high levels of coliforms in other tropical settings. Nevertheless, in this study, Colilert provided an easy and accurate assessment of water quality. Because the Colilert method is easy to use (Edberg *et al.* 1988, 1990; Edberg & Edberg 1988; Covert *et al.* 1989; Cowburn *et al.* 1994; Eckner 1998; Schets *et al.* 2002), it could be an alternative to membrane filtration or multiple tube fermentation in the setting of a developing country laboratory.

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