Household Microbial Water Quality Testing in a Peruvian Demographic and Health Survey: Evaluation of the Compartment Bag Test for *Escherichia coli*

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Abstract. The Joint Monitoring Program relies on household surveys to classify access to improved water sources instead of measuring microbiological quality. The aim of this research was to pilot a novel test for *Escherichia coli* quantification of household drinking water in the 2011 Demographic and Health Survey (DHS) in Peru. In the Compartment Bag Test (CBT), a 100-mL water sample is supplemented with chromogenic medium to support the growth of *E. coli*, poured into a bag with compartments, and incubated. A color change indicates *E. coli* growth, and the concentration of *E. coli*/100 mL is estimated as a most probable number. Triplicate water samples from 704 households were collected; one sample was analyzed in the field using the CBT, another replicate sample using the CBT was analyzed by reference laboratories, and one sample using membrane filtration (MF) was analyzed by reference laboratories. There were no statistically significant differences in *E. coli* concentrations between the field and laboratory CBT results, or when compared with MF results. These results suggest that the CBT for *E. coli* is an effective method to quantify fecal bacteria in household drinking water. The CBT can be incorporated into DHS and other national household surveys as a direct measure of drinking water safety based on microbial quality to better document access to safe drinking water.

INTRODUCTION

An estimated 748 million people worldwide lack access to safe drinking water sources, putting them at risk for waterborne illnesses.¹ The Millennium Development Goals of the United Nations set a target to halve the number of households lacking sustained access to safe water by the year 2015 and Post-2015 Sustainable Development Goals (SDGs) hope to ensure availability and sustainable management of water and sanitation for all. However, the Joint Monitoring Program (JMP) of the United Nations has no reliable basis to track progress toward the water targets due to the unavailability of simple and affordable methods to directly test the microbial quality of household drinking water. Instead, the JMP uses household surveys to determine whether the source of drinking water supply is either improved or unimproved. According to this simple classification system, global access to improved water sources increased between 1990 and 2015, and Millennium Development Goal Target 7c was considered to be “on track” and actually met in 2010, 5 years ahead of schedule.¹

However, evidence from United Nations Children’s Fund surveys indicated that in multiple countries one-half of protected dug wells and one-third of protected springs and boreholes were microbially contaminated.² Other evidences, including the Rapid Assessment of Drinking-Water Quality Projects and global modeling projections based on this data, have also called into question the microbiological safety of many water sources classified as improved under the definitions used by the JMP.³ Furthermore, there continue to be regional disparities in safe water access.^{1,2} To

decrease the lack of access to safe drinking water and disparities in access, it is necessary to identify microbially unsafe drinking waters to take actions improve them.^{4,5} Hence, simple, portable, and accessible tests to quantify the microbial quality of household drinking water in the field are much needed. The Compartment Bag Test (CBT) was created to meet this need.

A portable and simple test for determining the microbiological safety of household drinking water has been developed and used in the field by the investigators of this study.^{6,7} The CBT detects and quantifies target bacteria, such as *E. coli* or H₂S-producing bacteria, in 100-mL volumes of water as a most probable number (MPN) estimate with a sensitivity and specificity of 94.9% and 96.6%, respectively.^{6,7} The CBT is a clear, sterile, polyethylene bag (Nasco) that was modified to provide five internal compartments totaling 100 mL. Using the CBT, the presence and concentrations of target fecal bacteria in water, food, and other environmental media can be determined, thereby making it possible to evaluate safe water access, identify unsafe water, and reliably track progress toward the water target of the Millennium Development Goals and the water target of the Post-2015 SDGs. The CBT quantifies and distinguishes the *E. coli* concentrations in 100-mL samples of drinking water corresponding to the World Health Organization (WHO) decimal categories of potential health risk, which are < 1 *E. coli*/100 mL and deemed safe or very low risk, 1–10 *E. coli*/100 mL and considered of intermediate risk and relatively safe, > 10–100 *E. coli*/100 mL and considered high risk and likely unsafe, and > 100 *E. coli*/100 mL, considered very high risk and unsafe.⁸ Such microbial data on water quality makes it possible to take actions to improve unsafe water quality and thereby reduce enteric infectious disease risks from waterborne exposure sources.

The purpose of this study was to evaluate the performance of the CBT in quantifying microbial safety of household drinking water when performed by Demographic and

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Health Survey (DHS) field survey staff in Peru and to document the potential for its use in microbial water quality monitoring within DHS field data collection. The study was done by DHS survey teams for three geographically distinct and representative regions of Peru. A technician trained to perform the CBT on drinking water samples collected from households was added to each of the three survey teams. The *E. coli* concentration in 100-mL samples of household drinking water was measured by the CBT performed by survey staff in the field and compared with the results from parallel analysis of the same water samples by trained analysts in reference laboratories using both the CBT and a standard membrane filtration (MF) method. The *E. coli* concentrations/100 mL as colony-forming units (CFU) by the MF method were statistically compared with the MPN results of the CBT method.

MATERIALS AND METHODS

During March–June 2011, 704 households were surveyed in three regions in Peru by trained field teams from the Instituto Nacional de Estadística e Informática (INEI): Lima (Pacific coast), Junín (Andes mountains), and Loreto (Amazon jungle). Different geographical regions were included in this study to test the robustness of the CBT method. Field DHS surveyors received a 2-day training on household water quality testing for *E. coli* using the CBT prior to deployment in the field. Consistent with the sampling methodology of the DHS, INEI randomly selected households that the field DHS teams would survey and collect a sample of household drinking water from.⁹ Consenting households provided three 100-mL drinking water samples collected by the method that would have been used to get a volume to drink and then transferring the water to sterilized, disposable sample collection bottles (IDEXX) containing sodium thiosulfate to neutralize any chlorine. The water samples were identified and tracked using a unique sample code that “masked” the identification of the households. Collected water samples were transported and stored at or below 10°C with ice packs until analysis.

A member of the DHS survey team used a portable free chlorine test kit (DPD method; Hach Chemical) to measure the free chlorine residual concentration (mg/L) of collected tap water samples as described in the DHS guidelines; and the CBT was used to analyze one of the 100-mL household drinking water samples for *E. coli* in the field. Two replicate 100-mL samples were transported to the reference laboratory and analyzed using the CBT and MF methods. Membrane filters (HA membrane filters, 47 mm, 0.45 µm; Millipore) were incubated overnight for 20–28 hours at 37°C on absorbent pads (Millipore) with the same *E. coli* liquid culture medium as used with the CBT (Hi-Media). *Escherichia coli* colonies were detected by their distinctive blue color as in Environmental Protection Agency (EPA) Method 1604. In instances where electricity was not available in the field, 38% of the samples conducted with the CBT ($N = 252$) were incubated at room temperature (26–33°C) for at least 30 hours; otherwise CBT samples were incubated in a modified electric incubator (ThinkGeek™) fitted with vent holes and heated by an alcohol burner to achieve a temperature of 36–38°C for 20–28 hours.¹⁰

Positive results for *E. coli* in the CBT were indicated by a distinctive blue color change within bag compartments of 56-, 30-, 10-, 3-, and 1-mL volumes. This color change resulted from the hydrolysis activity of the β -glucuronidase enzyme unique to *E. coli* acting on the chromogenic beta-D-glucuronide substrate, indoxyl-beta-D-glucuronide (X-Gluc), in the medium. The concentration of *E. coli* in each compartment bag was calculated using the U.S. EPA MPN calculator, which uses the volumes of the positive and negative compartments to estimate an MPN/100 mL.^{11,12} The CBT has an uncensored, discrete upper detection limit of 48.3 *E. coli* MPN/100 mL of undiluted water. However, the censored upper limit based on all five compartments of the CBT turning positive should be 100 *E. coli* MPN or more/100 mL for conservative risk decision-making and calculation purposes. All *E. coli* concentration results were censored at an upper detection limit of 101 *E. coli*/100 mL for analysis.

Water quality data were transferred to Microsoft Excel for analysis. All data were entered twice to minimize data entry error. The data were then subjected to statistical analysis (GraphPad Prism 5, GraphPad Software, Inc., 2009; Stata 10.1, StataCorp, College Station, TX; SAS 9.3, Cary, NC) to determine the extent of agreement in measured concentrations of *E. coli* in household water samples as measured by¹ the DHS survey teams using the CBT,² the reference laboratories using the CBT, and³ the reference laboratories using a standard MF test. Data analysis used nonparametric matched pairs analyses and the Friedman test to determine the extent of agreement of the three different *E. coli* measurements of the same water sample. A paired *t* test or a nonparametric equivalent was used to determine whether the CBT test done by the survey team and done by the reference laboratory on the same water samples gave consistent results. The a priori threshold for statistical significance was $\alpha = 0.05$.

RESULTS

***Escherichia coli* in household drinking water samples.** Most water samples were analyzed for *E. coli* within 24 hours of sample collection. Samples for which holding times were excessive by being > 48 hours, of which there were 39 (5% of the total samples), were excluded from all statistical analyses.¹³ Across all three survey regions, the arithmetic average of *E. coli* concentrations found by field CBT, laboratory CBT, and laboratory MF were 16.8–17.5 *E. coli*/100 mL and had overlapping 95% confidence limits (Figure 1). The highest concentrations of *E. coli* in household drinking water samples were found in Loreto (Amazon jungle region) at > 101 CFU/100 mL, and the lowest in Lima (Pacific coast region) at < 0 CFU/100 mL. Within each region, *E. coli* concentrations measured in the field and laboratory by the CBT and measured in the laboratory by MF as arithmetic means were comparable and had overlapping 95% confidence limits (Figure 1). Geometric means were also calculated and compared and shown in Figure 2.

The statistical comparison for all three survey locations combined and separated by site is shown in Table 1. Because the data on *E. coli* concentrations did not conform to a normal probability distribution, nonparametric statistical methods were used. For the 665 household water samples

FIGURE 1. Arithmetic means and 95% confidence intervals for *Escherichia coli* concentrations in household drinking water samples by each test, and by each survey region—Lima ($N = 252$), Loreto ($N = 181$), and Junin ($N = 232$).

of all regions combined, there were no statistically significant differences in results for measured concentrations of *E. coli* between the reference laboratory (MF and CBT) and field analyses (CBT) by Friedman test (nonparametric repeated measures analysis of variance, $P = 0.25$). Likewise, Wilcoxon matched-pairs signed-ranks tests showed that there were no statistically significant differences in measured *E. coli* concentrations of the same household water samples of all survey locations combined between the laboratory and field CBT results ($P = 0.50$, Spearman's $r = 0.88$), between the laboratory CBT and laboratory MF

TABLE 1

Summary of matched pair nonparametric test results and Spearman's rank correlations for *Escherichia coli* concentrations in household water samples of all three survey locations combined and separated by site

Comparison	<i>P</i> value for either Friedman test for nonparametric repeated measures ANOVA† or for Wilcoxon matched-pairs signed-ranks tests†	Spearman's rank correlation (<i>R</i>)*
All locations	0.25†	0.88
Field CBT vs. laboratory CBT	0.50‡	0.80
Field CBT vs. laboratory MF	0.84‡	0.76
Laboratory CBT vs. laboratory MF	0.43‡	0.88
Lima (coast)		
Field CBT vs. laboratory CBT	0.68‡	0.81
Field CBT vs. laboratory MF	0.07‡	0.60
Laboratory CBT vs. laboratory MF	0.14‡	0.62
Loreto (jungle)		
Field CBT vs. laboratory CBT	0.29‡	0.90
Field CBT vs. laboratory MF	0.37‡	0.88
Laboratory CBT vs. laboratory MF	0.03‡	0.95
Junin (mountains)		
Field CBT vs. laboratory CBT	0.76‡	0.61
Field CBT vs. laboratory MF	0.16‡	0.60
Laboratory CBT vs. laboratory MF	0.08‡	0.88

ANOVA = analysis of variance; CBT = compartment bag test; MF = membrane filtration.
*Bold values indicate significant pairings.

results ($P = 0.43$, Spearman's $r = 0.88$), or between the field CBT and laboratory MF results ($P = 0.84$, Spearman's $r = 0.76$). In Loreto, which represented 38% of the total samples, electricity was unreliable and the ambient temperature was always above 25°C during the survey period; therefore, only ambient temperature incubation was used ($N = 232$). However, there was no significant difference found in *E. coli* concentrations as measured in the field by CBT versus

FIGURE 2. Geometric means of *Escherichia coli* concentrations in household drinking water samples by each test, and by each survey region—Lima ($N = 252$), Loreto ($N = 181$), and Junin ($N = 232$).

TABLE 2

Escherichia coli occurrence and concentrations for household drinking water samples with different concentrations of free chlorine

Chlorine concentration (mg/L)	Number of samples*	Percentage of samples negative for <i>E. coli</i>	<i>E. coli</i> arithmetic mean (MPN/100 mL) (95% CLs)	<i>E. coli</i> geometric mean (MPN/100 mL) (95% CLs)*	<i>E. coli</i> /100 mL (minimum, maximum)
≥ 0.5	120	84	4.4 (1.0–7.8)	0.19 (0.14–0.26)	0, > 101
0.1 to < 0.5	98	76	15.3 (8.2–22.4)	0.41 (0.25,0.68)	0, > 101
< 0.1	484	67	18.7 (15.5–22.0)	0.62 (0.49,0.80)	0, > 101

CL = confidence limit; MPN = most probable number.

*Fifty-nine samples did not have chlorine residual concentrations because the water was taken from a nontreated source, was bottled water, or the test could not be performed.

An MPN concentration of 0.1/100 mL was assigned to all samples in which no *E. coli* were detected (recorded initially as < 1 MPN/100 mL) to be able to calculate geometric mean concentrations.

by CBT or MF in the laboratory in Loreto despite use of ambient incubation.

In addition, analysis by individual survey region indicated that there were no statistically significant differences between the field and laboratory test results for *E. coli* concentrations in household water samples of Lima (Pacific coast), Loreto (Amazon jungle), or Junín (Andes mountains). However, the matched pair results between laboratory CBT and laboratory MF in Loreto (Amazon jungle) survey region were significantly different ($P = 0.03$) from each other using the Wilcoxon matched-pairs signed-ranks tests, but the Spearman's rank correlation ($R = 0.95$) shows that the values for *E. coli* in household water samples are similar and trending in the same direction.

***Escherichia coli* occurrence and concentrations in relation to residual chlorine.** The differences in the percentage of household water samples that were positive for *E. coli* categorized according to their chlorine concentrations were statistically significant by Pearson χ^2 test ($P = 0.001$) (Table 2). Households with > 0.5 mg/L free chlorine in sampled water had the highest percentage of samples negative for *E. coli* at 84%. However, 16% of these household water samples ($N = 19$) with > 0.5 mg/L free chlorine were positive for *E. coli*. When all samples were included in the analysis (including those with *E. coli* nondetects assigned an *E. coli* concentration of 0.1 MPN/100 mL), arithmetic and geometric mean *E. coli* concentrations were lowest in household water with ≥ 0.5 mg/L free chlorine (4.4 MPN/100 mL and 0.19 MPN/100 mL, respectively), and then increased as chlorine concentration decreased. Based on a Kruskal–Wallis test, concentrations of *E. coli* for each category of chlorine concentration in household water samples were found to be significantly different ($P = 0.0003$).

***Escherichia coli* concentrations according to water source and treatment.** Water sources classified as improved or unimproved were examined for *E. coli* occurrence and decimal concentration categories defined by the WHO,

based on CBT field results and MF laboratory results (Tables 3 and 4).

The number of samples in the different *E. coli* concentration categories depending on whether measured in the field by CBT or laboratory by MF categories was compared with Wilcoxon matched-pairs signed-ranks tests. For improved water sources, the number of samples in the different *E. coli* concentration categories based on field CBT results and laboratory MF results were not significantly different ($P = 0.969$). Likewise, for unimproved water sources, the number of samples in the different *E. coli* concentration categories based on field CBT results and laboratory MF results were also not significantly different ($P = 0.504$). These results further document that field CBT results are not statistically different to laboratory MF results in providing information about the safety of water based on *E. coli* decimal concentration categories.

Water sources classified as improved or unimproved based on JMP definitions as determined by the DHS survey were also examined for *E. coli* presence based on CBT field results and MF laboratory results (Tables 5 and 6) and no statistical difference was found between the two test methods using McNemar's test for improved sources ($P = 0.1638$) and unimproved sources ($P = 0.221$).^{1,2}

DISCUSSION

Based on the current classification system that identified drinking water as either improved or unimproved, available data suggests that the Millennium Development Goal target to improve access to "safe" water has already been achieved based on the proxy of improved versus unimproved drinking water sources.¹ However, the results of previous studies indicate that household drinking water samples from improved sources contained measurable concentrations of *E. coli* bacteria in 100-mL sample volumes.^{3–5} Therefore, a more objective and rational method based on microbial water quality

TABLE 3

Number and percentage of field CBT-analyzed household drinking water samples in each WHO decimal category of *Escherichia coli* concentration for improved and unimproved sources

Field CBT results, <i>E. coli</i> /100 mL	Number (%) of households		
	Improved	Unimproved	Total
< 1	353 (74)	125 (70)	519 (74)
1–10	43 (9)	13 (7)	56 (8)
> 10–100	27 (5)	15 (8)	44 (6)
> 100	58 (12)	26 (15)	85 (12)
Total	481 (100)	179 (100)	704 (100)

CBT = compartment bag test; WHO = World Health Organization.

TABLE 4

Number and percentage of laboratory MF-analyzed household drinking water samples in each WHO decimal category of *Escherichia coli* concentration for improved and unimproved sources

Laboratory MF results, <i>E. coli</i> /100 mL	Number (%) of households		
	Improved	Unimproved	Total
< 1	386 (80)	140 (78)	570 (81)
1–10	35 (7)	21 (12)	56 (8)
> 10–100	57 (12)	18 (10)	75 (11)
> 100	3 (1)	0 (0)	3 (0)
Total	481 (100)	179 (100)	704 (100)

MF = membrane filtration; WHO = World Health Organization.

TABLE 5

Number of *Escherichia coli* detects and nondetects for MF and CBT for improved sources

		MF		Total
		Detects (% of total)	Nondetects (% of total)	
CBT	Detects	95 (19.8)	21 (4.4)	116
	Nondetects	12 (2.5)	353 (73.4)	365
	Total	107	374	481

CBT = compartment bag test; MF = membrane filtration.

data is needed to better characterize water supplies as safe or unsafe to better manage drinking water safety; the JMP along with other organizations that evaluate progress toward the water target of the Post-2015 SDGs should focus on how to perform systematic microbiological water quality testing of household water samples on a national scale in all countries.

In this study, measurable concentrations of *E. coli* bacteria were found in water samples with measurable chlorine residuals. This finding may seem implausible because of the widespread use of chlorine to disinfect drinking water and achieve nondetectable levels of fecal bacteria in 100-mL sample volumes. However, *E. coli* have been detected previously in drinking water samples with measurable chlorine residuals.¹⁴ The presence of detectable levels of *E. coli* and other fecal bacteria in drinking water samples with detectable free chlorine residual could be caused by the presence of injured but culturable fecal bacteria in chlorinated waters.¹⁴ Other possible explanations for the presence and persistence of such bacteria in drinking water with measureable chlorine residuals is the presence of fecal bacteria in aggregates, protection within association biofilms, or insufficient time for inactivation if contamination was recent. Another possible explanation for *E. coli* detection in household drinking water samples even when free chlorine was present in household drinking water samples is that these water samples were taken from different water containers or sources used by the household or at different times. For example, 18 of these 19 households said that they stored drinking water. It is possible that the water sample used to measure *E. coli* was taken from the storage container but the sample for free chlorine analysis was perhaps taken directly from the tap, according to the established guidance in the DHS manual.⁹

The CBT is a promising tool that provides an opportunity to do widespread and routine microbiological testing of household drinking water on samples collected during household surveys. The CBT has also potential application

for local water quality monitoring, such as water surveillance monitoring by district health offices. The CBT is simple, portable, and performs similarly to other more complex, less portable, and more expensive tests that are unsuitable for field use in low-resource settings.¹⁵ Many of these tests must be done in specialized laboratories by skilled technicians.^{6,7,15} When taking into account the cost of running specialized laboratories, the CBT is cost competitive, about 5–10 USD/sample, and its exact cost depends on the bacteriological medium used to detect the target fecal bacteria of interest.^{6,15,16} The CBT is particularly advantageous because it does not require electricity or supplemental equipment other than an incubator where ambient temperatures are unsuitable to grow *E. coli* bacteria. The CBT allows water quality monitoring in a variety of environments; water quality monitoring is important in overcoming urban/rural disparities and achieving progressive realization of the human right to safe water.^{4,5} The agreement between the field and laboratory data on *E. coli* concentrations in household water samples suggests that the CBT for *E. coli* is an effective method to quantify bacteria of fecal origin in household drinking water overall and is especially amenable to field use. Also, no statistical difference was found between the ambient temperature results of the CBT as performed by field survey workers compared with CBT results obtained by the reference laboratories using standard incubation conditions on the same household water samples.

One of the limitations of the CBT in its applicability to some types of water samples is its uncensored upper detection limit of 48.3 *E. coli* MPN/100 mL of undiluted water. Many ambient environmental waters can have concentrations > 100 *E. coli*/100 mL, including those approved for primary contact recreation, such as swimming.¹⁷ However, this upper limit concentration of 100 *E. coli*/100 mL greatly exceeds the recommended *E. coli* level of the WHO Guidelines for Drinking-water Quality which is none detectable/100 mL.⁸ Therefore, the CBT provides actionable information for decision-making about the safety of drinking water because it quantifies and distinguishes the *E. coli* concentrations in 100-mL samples of drinking water corresponding to the WHO decimal categories of potential health risk, which are < 1 and deemed safe or very low risk, 1–10 and considered of intermediate risk and relatively safe, > 10–100 and considered high risk and likely unsafe, and > 100, considered very high risk and unsafe.⁸

In situations where all compartments turn positive, indicating exceedance of the highest concentration of bacteria detectable by the CBT and a high-risk likelihood of containing 100 or more *E. coli*/100 mL, the possibility that the *E. coli* concentration is actually much higher than this value should be considered with regard to the human health risks from exposure. For applications of the CBT to waters expected to have higher concentrations of *E. coli*, it is readily possible to compensate for the lower upper detection limit by first appropriately diluting the water sample in *E. coli*-free dilution water perhaps 10-fold or more, as has been reported previously for the use of the CBT to analyze a range of different ambient water samples in Atlanta, GA.⁷

Another limitation of the CBT is that it has only five compartments. This limited number of sample subvolumes results in somewhat broader confidence interval estimates of MPN concentration of bacteria than those of other MPN

TABLE 6

Number of *Escherichia coli* detects and nondetects for MF and CBT for unimproved sources

		MF		Total
		Detects (% of total)	Nondetects (% of total)	
CBT	Detects	54 (30.2)	5 (2.8)	59
	Nondetects	1 (0.6)	119 (66.5)	120
	Total	55	124	179

CBT = compartment bag test; MF = membrane filtration.

tests that use a greater number of discrete sample volumes. However, the upper 95% confidence limit values of MPN values of the CBT are not so large in magnitude relative to the MPN concentration estimates that the water would be classified differently on the basis of the WHO decimal categories of *E. coli* concentration/100 mL.

This study shows that the CBT to quantify *E. coli* concentration in 100-mL volumes in drinking water can be incorporated into a DHS to determine the quality of household drinking water and provide results consistent to those obtained on the sample water samples using a standard *E. coli* quantification test performed by trained analysts in a standard analytical laboratory. Field DHS surveyors described the CBT as simple to use and interpret, and lightweight to carry. Furthermore, the ability of the CBT to be done successfully under challenging field conditions in diverse geographic regions of Peru highlights the robustness and applicability of the test in a wide range of settings. The successful incorporation of the CBT within the Peruvian DHS provides evidence that should encourage its use for analysis of field household and community drinking water samples to support the JMP-proposed water quality targets for the Post-2015 SDGs, which includes measurement of *E. coli* concentrations as a fecal indicator of drinking water. Further field evaluation of the CBT is recommended to more fully determine its validity and field applicability in *E. coli* detection. However, the results of this study demonstrate the ability of the CBT to be reliably and effectively performed in the field by DHS staff and give results comparable to a widely used standard *E. coli* test performed by trained and experienced analysts in a typical microbiology laboratory.

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REFERENCES

1. World Health Organization/United Nations Children's Fund, 2014. *Progress on Sanitation and Drinking Water: 2014 Update*. Geneva, Switzerland: World Health Organization.
2. World Health Organization/United Nations Children's Fund, 2010. *Progress on Sanitation and Drinking Water: 2010 Update*. Geneva, Switzerland: World Health Organization.
3. Onda K, LoBuglio J, Bartram J, 2012. Global access to safe water: accounting for water quality and the resulting impact on MDG progress. *Int J Environ Res Public Health* 9: 880–894.
4. Bain R, Cronk R, Hossain R, Bonjour S, Onda K, Wright J, Yang H, Slaymaker T, Hunter P, Pruss-Ustun A, Bartram J, 2014. Global assessment of exposure to faecal contamination through drinking water based on a systematic review. *Trop Med Int Health* 19: 917–927.
5. Shaheed A, Orgill J, Montgomery M, Jeuland M, Brown J, 2014. Why “improved” water sources are not always safe. *Bull World Health Organ* 92: 229–308.
6. McMahan L, Devine A, Grunden A, Sobsey M, 2011. Validation of the H₂S method to detect bacteria of fecal origin by cultured and molecular methods. *Appl Microbiol Biotechnol* 92: 1287–1295.
7. Stauber C, Miller C, Cantrell B, Kroell K, 2014. Evaluation of the compartment bag test for the detection of *Escherichia coli* in water. *J Microbiol Methods* 99: 66–70.
8. World Health Organization, 2004. *Guidelines for Drinking-Water Quality*. Geneva, Switzerland: World Health Organization, 392–394.
9. Demographic and Health Surveys, 1996. *Sampling Manual DHS-III Basic Documentation No. 6*. Calverton, MD: Macro International Inc.
10. Brown J, Stauber C, Murphy J, Khan A, Mu T, Elliott M, Sobsey M, 2011. Ambient-temperature incubation for the field detection of *Escherichia coli* in drinking water. *J Appl Microbiol* 110: 915–923.
11. Cochran WG, 1950. Estimation of bacterial densities by means of the “most probable number.” *Biometrics* 6: 105–116.
12. Klee AJ, 1993. A computer program for the determination of most probable number and its confidence limits. *J Microbiol Methods* 18: 91–98.
13. U.S. Environmental Protection Agency (USEPA), 2008. *Addendum to New Microbiology Test Methods for E. coli*. Washington, DC: USEPA. Available at: <http://www.cdph.ca.gov/certific/lab/Documents/ELAPAddendMicro.pdf>.
14. McFeters G, Camper A, 1983. Enumeration of indicator bacteria exposed to chlorine. *Adv Appl Microbiol* 29: 177–193.
15. Bain R, Bartram J, Elliott M, Matthews R, McMahan L, Tung R, Chuang P, Gundry S, 2012. A summary catalogue of microbial drinking water tests for low and medium resource settings. *Int J Environ Res Public Health* 9: 1609–1625.
16. Crocker J, Bartram J, 2014. Comparison and cost analysis of drinking water quality monitoring requirements versus practice in seven developing countries. *Int J Environ Res Public Health* 11: 7333–7346.
17. Brenniman GR, Rosenberg SH, Northrop RL, 1981. Microbial sampling variables and recreational water quality standards. *Am J Public Health* 71: 283–289.