

Comparison and verification of four field-based microbiological tests: H₂S test, Easygel[®], Colilert[®], Petrifilm[™]

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ABSTRACT

The UN defines water supplies as 'improved' or 'unimproved.' These indicators are easy to measure, but do not reflect water quality, which requires laboratory or field tests. Laboratory and test availability, expense and technical capacity are obstacles for developing countries.

This research compares and verifies four low-cost, field-based microbiological tests: the EC-Kit (Colilert[®] and Petrifilm[™] tests), the H₂S bacteria test, and Easygel[®], against a standard method (Quanti-Tray[®]). The objectives are to: (1) verify the accuracy of the four field-based tests, (2) study the accuracy of these tests as a function of improved and unimproved sources; (3) recommend a single microbiological test, if appropriate, based on accuracy and cost, and/or (4) recommend a testing combination, if appropriate, based on accuracy and cost.

The tests of 500+ total water samples from Capiz Province, Philippines and Cambridge, MA indicate that two-tests systems gave better results than a single test. Both the 100-mL H₂S test + Petrifilm[™] and the 20-mL H₂S test + Easygel[®] combinations yield promising results, in addition to being inexpensive. None of the field-based tests should be used on their own. We recommend further verification of a larger sample size and scale be undertaken before these testing combinations are recommended for wider use.

Key words | Colilert[®], drinking water, Easygel[®], H₂S, Petrifilm[™], Quanti-Tray[®]

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INTRODUCTION

The United Nations (UN) describes water as 'indispensable for leading a life of human dignity' and 'a prerequisite for the realization of other human rights'. Still, 13% of the world's population, or 884 million people, lack access to an improved drinking water source (WHO/UNICEF 2010) and, every year, at least 1.8 million people – the majority of them children under the age of five (WHO/UNICEF 2010) – die from diarrhoeal diseases related to unsafe water, sanitation and hygiene.

The UN has therefore developed Millennium Development Goal Target 7C, which aims to halve the proportion of people without sustainable access to adequate water and sanitation by the year 2015 (United Nations 2000). The indicators used for these numbers include the

proportion of the population that uses an improved drinking water source, and the proportion that uses an improved sanitation facility (United Nations 2000). The ideal solution to this problem is to provide a drinking water supply that is both reliable and safe. According to the UN, access to drinking water means that the source is less than one kilometre away from its place of use, and that it is possible to reliably obtain at least 20 litres of water per member per household per day. The UN also defines safe drinking water as water with microbial, chemical and physical characteristics that meet World Health Organization (WHO) guidelines or national standards on drinking water quality (United Nations 2000).

Although the accessibility and reliability of a drinking water source are relatively easy to ascertain, determining the safety of a drinking water source requires the performance of

drinking water quality tests. The UN currently defines sources as 'improved' or 'unimproved', which does not necessarily reflect the actual quality of the drinking water. However, laboratory testing is especially difficult in developing countries where funds, technology, laboratory facilities and trained personnel are in short supply. Also, water quality testing raises even more questions: What contaminants should the drinking water be tested for and what test(s) should be used? Which sources should be sampled, how many of them should be sampled, and how often?

Although there are many types of drinking water contamination, this study will solely focus on microbiological contamination of drinking water, which occurs when drinking water is contaminated at the source by human or animal faeces, or through inappropriate transportation, handling or storage in vessels in the household. This research aims to verify and compare the accuracy of four field-based tests (10-mL pre-dispensed Colilert[®], 3M[™] Petrifilm[™], hydrogen sulfide (H₂S) bacteria test and Easygel[®]) against a standard method (Quanti-Tray[®]). The drinking water sources tested were located in Capiz Province, Philippines, and in Cambridge, Massachusetts.

Project area

The Philippines is an archipelago made up of more than 7000 islands, located in Southeast Asia. The population of the Philippines is estimated at almost 98 million as of July 2009, making it the 12th most populated country in the world. The country has a high rate of food- and water-related diseases such as bacterial diarrhoea, hepatitis A, typhoid fever, dengue, malaria and Japanese encephalitis, which is worsened by the tropical marine climate (CIA 2009).

Capiz Province is situated on the northeastern part of Panay Island, located in the Western Visayas. It has a land surface area of approximately 2600 km² and has roughly 80 km of coastline. In 2007, the population of Capiz was estimated at approximately 701,000 with 148,000 people living in the capital, Roxas City. The province is divided into 17 areas: 16 municipalities and Roxas City.

Project initiation

Until 2009, Capiz had never performed any testing of the various drinking water sources (wells, springs, surface

water and piped supplies) used throughout the province, with the exception that the Roxas City municipal water treatment plant sent treated water samples to a laboratory on another island for regular testing. During Fall 2008, Dr Jarvis Punsalan, MD, MPH, Director of Public Health (DPH) head of the Capiz Province Provincial Health Office (PHO), received funding from the European Commission, the Philippines' government's Department of Health and United Nations Children's Fund (UNICEF) to set up a water quality testing laboratory at Roxas Memorial Hospital, in Roxas City, which would test for microbiological contamination. He contacted Susan Murcott, Senior Lecturer at the Massachusetts Institute of Technology (MIT), for advice on the types of microbiological drinking water quality tests to conduct, and she recommended two types of tests: IDEXX Quanti-Tray and the EC-Kit. These tests will be described more fully in the following sections.

During 2009, Capiz's PHO purchased EC-Kits and Quanti-Tray tests. An incubator, ultraviolet (UV) light and Quanti-Tray sealer were also purchased in order to conduct the Quanti-Tray tests. In May 2009, a Filipino non-profit organization, 'A Single Drop', trained the Capiz PHO staff, municipal health officers and sanitary inspectors (SIs) on how to sample water sources, use the EC-Kit and interpret the sample results. The Quanti-Tray equipment finally arrived in November 2009 and, as part of that purchase, the laboratory staff of the PHO's Roxas City office received training from the suppliers in the set-up and use of the Quanti-Tray system. During October to December 2009, in collaboration with the MIT team, the PHO developed a water quality assessment survey designed to test 1000 different water supplies from all 16 municipalities and Roxas City, which took place from December 2009 to March 2010. In addition, the H₂S and Easygel tests were suggested as potential complementary tests to the EC-Kit, to be verified during the Capiz Province water quality testing program. This water quality assessment survey would be the first-ever comprehensive drinking water quality testing in the province.

The main PHO participants in this project included Dr Jarvis Punsalan, MD, DPH, head of the Capiz PHO; Jane Delos Reyes, Engineer, coordinator of the water quality testing program; Leo Biclar, medical technician responsible for processing and interpreting the Quanti-Tray tests; and SIs at

the provincial and municipal levels who were in charge of collecting the water samples and processing and interpreting the EC-Kit tests.

Objectives of study

The objectives of this study are to: (1) verify the accuracy of the four field-based tests: 10-mL pre-dispensed Colilert, Petrifilm, H₂S bacteria test (laboratory-made reagent in 10-, 20- and 100-mL sample volume; and the industry-made HACH reagent with 20-mL sample volume) and Easygel against a standard method: Quanti-Tray, using STATA[®] statistical software; (2) study the accuracy of the four field-based tests as a function of improved and unimproved sources; (3) if it is determined that field-based microbiological tests are accurate, provide recommendations for the use of a single test based on accuracy and cost; and/or (4) provide recommendations for the use of a testing combination, if appropriate, based on accuracy and cost.

Drinking water sources and types

The WHO Joint Monitoring Programme (JMP) for Water Supply and Sanitation has been assembling statistics on drinking water and sanitation coverage since 1990. Since 2000, the JMP has classified water sources as 'improved' or 'unimproved' (WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation 2005). The overall assumption behind the improved/unimproved drinking water source categories is that improved sources are more likely to provide safe water than unimproved sources. The Drinking Water Source Classification is presented in Table 1.

According to the Philippines' government's regulatory definitions, 'improved' water sources include three levels: Level 1, which consists of point sources, Level 2, which consists of communal faucet systems and Level 3, which consists of piped systems with individual household connections. The unimproved water sources, referred to as 'doubtful sources' in the Philippines, consist of open dug wells, unimproved springs, rainwater and surface water sources. According to the National Statistical Coordination Board (NSCB) of the Philippines, as of 2000, 119,000 households in Capiz (or 92% of the Capiz population) had access

Table 1 | WHO drinking water source classification

Improved sources of drinking water	Unimproved sources of drinking water
Piped water into dwelling, yard or plot	Unprotected dug well
Public tap/standpipe	Unprotected spring
Tubewell/borehole	Vendor-provided water
Protected dug well	Tanker truck water
Protected spring	Surface water (river, stream, dam, lake, pond, canal, irrigation channel)
Rainwater collection	Bottled water*

* Bottled water is considered an 'improved' source of drinking water only where there is a secondary source that is 'improved'.

to a UN-designated 'improved' drinking water supply (NSO 2002). A summary of the Capiz Province and corresponding UN designation are presented in Table 2.

METHODS

Research design

In December 2009, the Capiz PHO began the 1000 test program covering 16 municipalities and Roxas City. As collaborators to this project, the authors travelled to and worked in Capiz for approximately 22 working days, beginning on January 7, 2010.

The research plan and methodology for this study were comprised of the following steps.

1. Elaboration of a study design concerning the villages/sampling zones to be tested by the MIT team and PHO SIs using the Quanti-Tray and EC-Kit tests. This study design was prepared by Punsalan and Reyes, in collaboration with Susan Murcott of MIT, Tom Mahin of the Massachusetts Department of Environmental Protection and the four-person Masters of Engineering student team from MIT.
2. Elaboration of a study design concerning the villages/sampling zones to be tested using the H₂S bacteria tests (laboratory-made reagent: 10-, 20- and 100-mL sample volume and industry-made reagent: HACH with 20-mL sample volume) and Easygel test. This study design was prepared by Trottier, in collaboration with Murcott.

Table 2 | Levels of drinking water sources in the Philippines

UN designation	Capiz PHO designation	Source types	Capiz Province population
Unimproved	Doubtful	Unimproved springs, open dugwells or wells that need priming, surface water, or rainwater collectors	8%
Improved	Level 1	Stand-alone point sources, including shallow wells, tubewells with handpumps	92%
	Level 2	Piped water supply with communal water points, from boreholes, tubewells	
	Level 3 (piped connection on premises)	Piped water supply with private water points, such as a household connection	

- Field testing in Capiz Province, Philippines, using the Quanti-Tray, EC-Kit (Colilert and Petrifilm), H₂S bacteria and Easygel tests. The field testing was undertaken by the Capiz Province PHO and municipal SIs in collaboration with the MIT team.
- Field testing of the Charles River in Cambridge, Massachusetts, using the Quanti-Tray, H₂S bacteria and Easygel tests. The field testing was undertaken in April 2010 by Chuang and Trottier.
- Statistical comparison of the four field-based tests (Colilert, Petrifilm, H₂S bacteria and Easygel tests) in order to verify their accuracy and provide recommendations concerning the suitability of these tests for determining microbiological contamination of drinking water.

The study design plan established by Punsalan and Reyes consisted of selecting one sampling zone for every 5000 population (e.g. a municipality with a population of 30,000 would have 6 sampling zones selected), where zones were distributed according to the ratio of water sources accessed by the residents of the particular municipality.

Water samples were then randomly selected: the names of qualified villages or zones per town were put in a box and drawn randomly with 25% additional names drawn as reserve in case of inaccessibility of those initially selected. Water source selection was based on accessibility and their use by at least ten nearby households in the sampling zone:

- For each selected zone having doubtful sources, five of these sources were randomly selected and tested.
- For each village randomly selected for Level 1 supply testing, five Level 1 water sources were randomly selected for testing.
- For each village randomly selected for Level 2 supply testing, one reservoir was randomly selected and five of its outlets were tested. Water sources tested were the reservoir outlets. A maximum of five outlets per reservoir were tested.
- For each village randomly selected for Level 3 supply testing, five households accessing water from these sources were randomly selected and tested per zone. Water sources tested were every tenth household within the zone until the needed number of samples (five) was attained.

The only exception to the aforementioned study design was the Level 3 water supply for Roxas City. Since all of Roxas City has a piped, chlorinated water supply, this was tested separately using chlorine residual testing instead of the costlier bacteriological testing. The Hach® Free Chlorine Pocket Colorimeter II Test Kit was used to test randomly selected sample locations. The 85 samples tested for chlorine residual showed poor compliance with the DOH standard of 0.2 to 0.5 mg/L of free chlorine in the distribution system.

The study design plan for H₂S and Easygel testing consisted of analysing a subset of all the water samples tested (by the Capiz PHO and municipal SIs) during the month of January 2010, by biasing the sample selection toward Doubtful or Level 1 sources and known contaminated sources.

Sample collection and testing

Drinking water samples were collected directly from the source or from the point-of-use by the authors or municipal SIs. In some circumstances where the point-of-use location was not sufficient for sampling (e.g. a storage tank that was no longer filled with water), then samples were collected from the household storage containers. To ensure

sterile conditions, water samples were collected into three separate containers: sterile 100-mL polystyrene vessels for Quanti-Tray laboratory testing, 100-mL sterile sampling bags for EC-Kit field testing and 300-mL sterile sampling bags for H₂S bacteria testing and Easygel testing.

Figure 1 summarizes the general water sampling and testing methodology used by the PHO and the authors in the field research. The water samples were identified using a labelling system created by members of the Capiz PHO.

Microbiological test methods

The two microbiological drinking water quality tests used for the PHO's water quality assessment program were Quanti-Tray and EC-Kit. In addition, the H₂S bacteria and Easygel tests were suggested as potential complementary tests to the EC-Kit, to be verified during the Capiz Province water quality testing program.

Quanti-Tray

The IDEXX Quanti-Tray and Quanti-Tray/2000 are enzyme-substrate coliform tests (Standard Methods 9223) that use semi-automated quantification methods based on Most Probable Number (MPN).

The MPN method uses multiple qualitative (presence/absence) data points to generate a maximum probability coliform count per 100-mL value, given by a standard MPN table. The Quanti-Tray provides bacterial counts (of

total coliform and *E. coli*) as low as 1 MPN/100 mL and up to 200.5 MPN/100 mL of sample, whereas the Quanti-Tray/2000 provides bacterial counts up to 2419 MPN/100 mL.

The Quanti-Tray can test both chlorinated and un-chlorinated samples. However, chlorinated samples should first be treated with sodium thiosulfate before the Quanti-Tray reagent is added.

The Quanti-Tray is easy-to-use, rapid and accurate, and has been approved by the United States Environmental Protection Agency (EPA) for drinking, source/surface, ground and wastewaters (IDEXX 2010). However, one of the main drawbacks of the Quanti-Tray is its cost, since Quanti-Tray requires the use of an expensive sealer, and the trays and reagents were particularly expensive (\$21/test in Capiz), which makes this method generally unaffordable in developing countries.

Inadvertently, the Quanti-Tray tests purchased by the Capiz PHO and used during the Capiz laboratory analyses were the regular 50-well Quanti-Tray, whereas the Quanti-Tray tests purchased at MIT and used during the laboratory studies were the Quanti-Tray/2000. In retrospect, it would have been more useful for the Capiz PHO to purchase the Quanti-Tray/2000 since many of the water samples tested using Quanti-Tray had results that were higher than the Quanti-Tray detection limit (200.5 MPN/100 mL). However, since the Capiz PHO was going to use the Quanti-Tray to test drinking water samples, there was no reason to suspect that so many water samples would go above the Quanti-Tray detection limit.

Colilert and Petrifilm (EC-Kit)

A portable microbiology testing kit was initially developed by Robert Metcalf, Professor of Microbiology at California State University at Sacramento and one of the original founders of Solar Cookers International. He introduced this method to Susan Murcott in Kenya in 2005 and conducted training at MIT in June 2008. Susan Murcott then modified the testing kit to also include a waist-belt incubator and other materials needed in order to perform and interpret the tests. The waist-belt incubator, which incubates water samples using body temperature alone, serves as a cheaper, portable and more convenient alternative to

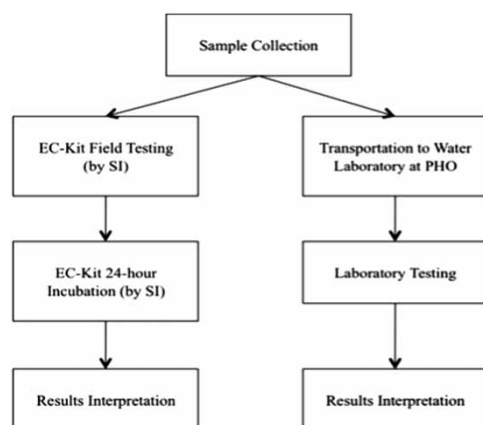


Figure 1 | Flow diagram representing sampling/testing methodology.

traditional incubators that are often costly and usually require electricity. She also created several different model sizes of the product and branded it with the name 'EC-Kit'. Susan Murcott introduced the technology to the Non-Governmental Organization (NGO) 'A Single Drop', and introduced the director of that NGO to Robert Metcalf, after which they brought the technology to the Philippines.

The EC-Kit contains two complementary tests for *E. coli*: the Colilert 10-mL presence/absence (P/A) test and Petrifilm test. The Colilert test contains the same formulation as in the Quanti-Tray tests, only it is reduced to its simplest form: a single P/A test of a 10-mL sample. However, the Colilert test has a detection limit equivalent to 10 MPN/100 mL. In the Colilert test, the substrate is hydrolyzed by the total coliform by-products, and reacts with a specific enzyme found in *E. coli*. A positive result is given by a yellow sample (presence of total coliforms), or a sample that fluoresces under long-wave UV illumination in the dark (presence of *E. coli*) after 24-hour incubation (Gerba 2000). The Petrifilm test provides a quantitative count of total coliform bacteria colonies (red colonies with gas bubbles after 24-hour incubation) and *E. coli* colonies (blue colonies with gas bubbles after 24-hour incubation) with a 1-mL sample volume.

Colilert can test both chlorinated and un-chlorinated samples. However, chlorinated samples should first be treated with sodium thiosulfate before the Colilert reagent is added. On the other hand, the Petrifilm cannot be used to test chlorinated samples.

The EC-Kit is simple, low cost and easy to use. The most promising features of the EC-Kit are that it can be used by virtually anyone who receives the brief 15- to 30-minute training, and bacterial incubation is performed using the waist-belt incubator, so it is completely portable.

H₂S test

The H₂S test using the original M1 medium is a well-known, simple and low-cost P/A test, developed by Manja *et al.* (1982). The test identifies the presence of H₂S-producing bacteria, associated with faecal contamination in a volume of water, which has been shown to correlate with the

presence of faecal contamination. Venkobachar *et al.* (1994) later developed a second test medium, M2, which consisted of the original M1 medium with the addition of L-cystine, which was shown to increase the sensitivity and reliability of the H₂S test (Pillai *et al.* 1999).

The M2 medium was used throughout the water quality testing program in Capiz Province in January 2010 for all sample sources and for all sample volumes (10-, 20- and 100-mL). Indeed, since the H₂S test reagent includes a chlorine-neutralizing compound (sodium thiosulfate), the H₂S test is a suitable microbiological test for chlorinated water supplies.

Another H₂S test used in this study was the industry-made HACH Pathoscreen. This test uses a powder-form, dehydrated H₂S test reagent, suitable for a 20-mL (or 100-mL) sample volume.

Easygel

The Easygel test is a quantitative water quality test that uses an enzyme substrate method to provide a total coliform and *E. coli* bacterial count present in a 0.5-mL to 5-mL sample volume, depending on the microbiological quality of the water sample tested.

The Easygel medium contains a sugar linked to different dyes that react with coliform and *E. coli* products to produce pink colonies (total coliform) and purple colonies (presence of *E. coli*) (Micrology Laboratories 2008).

Easygel can be used to test both chlorinated and un-chlorinated samples, without the addition of sodium thiosulfate.

One of the main advantages of the Easygel test is that it serves as an agar replacement. Agar is difficult to prepare, requires specific reagents and equipment, and preparation is both labour and time consuming. However, the Easygel sample processing procedure is very simple: 0.5 mL to 5 mL of the water sample is pipetted into the Easygel reagent bottle and the resulting mixture is poured into the pre-treated petri dish and allowed to set for 30 minutes. A sample volume of 5 mL was used for all Easygel samples in Capiz and in Cambridge, Massachusetts.

An added benefit of the Easygel test is that if an electric incubator is not available, samples can be incubated at ambient temperature (Micrology Laboratories 2009). So Easygel is

an economical, hassle-free and portable alternative, which makes it convenient for field use, in developing countries.

RESULTS AND DISCUSSION

A total of 521 water samples were collected in Capiz Province (the overall test program was of 1000 sources. We report here on a total of 521 water samples, a subset of the overall work, which was not yet completed at the time of this report). Each water sample was tested in the field using at least two tests: one field-based test (Colilert, Petrifilm, H₂S bacteria test and Easygel) and the standard method (Quanti-Tray). In Capiz, 521 samples were tested using the EC-Kit tests (Colilert and Petrifilm), 163 samples were tested using the H₂S bacteria test and 43 samples were tested using the Easygel test. Furthermore, 40 water samples were also collected in Cambridge, Massachusetts, in order to provide additional comparative data for the H₂S bacteria tests (10-, 20- and 100-mL sample volume) and Easygel test.

In total, 521 samples were tested using the EC-Kit tests; 204 samples were tested using the H₂S bacteria tests; and 83 samples were tested using the Easygel test. It is important to note that although both total coliform and *E. coli* were tested, we report here on *E. coli* contamination only because total coliform is not necessarily from the faeces of humans and mammals and some coliform can occur naturally in the environment. Therefore, total coliform is not the ideal indicator of faecal contamination (in contrast, *E. coli* and most thermotolerant coliform are). Therefore, *E. coli* is the most widely used test to determine whether there is human or animal faeces in drinking water.

Statistical analyses

The data presented here is a compilation of the data collected in Capiz Province from December 2009 to March 2010. The statistical analysis for Capiz Province results compares the field-based microbiological tests (Colilert, Petrifilm, H₂S test and Easygel) to Quanti-Tray. All statistical results presented here were analysed using the STATA Release II software.

The four new, field-based tests (and combinations) were analysed using contingency tables, general statistical analyses (True Results (TR), False Positives (FP) and False Negatives (FN)) and by calculating the error and proportional reduction in error. More information on each of these tests is provided below.

Frequency distribution table

A 2×2 frequency distribution table (Table 3) is a table used in bi-variate analyses and is composed of two rows, cross-classified by two columns. It is often used to display data that can be classified by two different variables (e.g. New Test and Standard Method), each of which has two possible outcomes, in this case Presence or Absence. Each of the four cells (*a*, *b*, *c* and *d*) represents the number of times the outcome falls within that cell.

Similarly, a 3×3 frequency distribution table is a table used in tri-variate analyses and is composed of three rows, cross-classified by three columns. They serve the same purpose as 2×2 frequency distribution tables; however, since enumerative tests give more information as to the degree of *E. coli* contamination, then a higher degree frequency distribution table can be set up with two different variables (e.g. New Test and Standard Method), each of which has multiple outcomes (WHO risk levels: Conformity, Low, Intermediate, High, Very High).

General statistical analyses

When a New Test is being compared against a Standard Method, the percentage of true results (TRs = $a + d$), false positives (FPs = b) and false negatives (FNs = c) is calculated.

These results provide information as to the 'correctness' of the given test (TR), and also specify the tendency of a test to incorrectly flag a positive result when it should be

Table 3 | Frequency distribution table

New test	Standard method	
	presence	Absence
Presence	<i>a</i>	<i>b</i>
Absence	<i>c</i>	<i>d</i>

negative, or to incorrectly flag a negative result when it should be positive.

Error and proportional reduction in error

The error associated with a given test is the sum of FP and FN results, divided by the total number of tests. The proportional reduction in error, λ , is a measure of ‘how good one becomes at making predictions’ starting from an initial test result prediction (with corresponding Error₁) and then adding another piece of information (in this case, a new, field-based test) to obtain a test result that will hopefully yield a better prediction (with corresponding Error₂). The formula for λ is provided below.

$$\lambda = \frac{Error_1 - Error_2}{Error_1}$$

In this case, the initial assumption was that UN-designated unimproved water sources (or Doubtful sources in the Philippines) were all contaminated (High/Very High Risk Level or Presence of contaminant), and that UN-designated improved water sources (or Levels 1 through 3 in the Philippines) were all safe (Conformity/Low Risk Level or Absence of contaminant). The error associated with the initial assumption was calculated: it was determined that for unimproved sources, the initial error was 15%; and for improved sources, the initial error was 63%. In other words, the assumption that an unimproved water source is contaminated would be incorrect 15% of the time, and the assumption that an improved water source is safe would be incorrect 63% of the time.

The calculations for error (Error₁ and Error₂) for tests and test combinations were done in two ways. The ‘exact match’ method reports the percentage frequency of tests results in the black-tone areas in the 2 × 2 and 3 × 3

Table 4 | Calculations for error for new, field-based tests

		Quanti-Tray	
		Presence	Absence
Water source type + additional test	Presence		
	Absence		

frequency distribution tables presented in Tables 4 and 5, respectively. In other words the black-tone areas in these two tables indicate an exact match and the hatch-marked areas represent an error. This is useful information for verification of the new field-based tests, but as a water quality test an overestimate of the risk level still yields useful information. So, the error values of interest actually follow the hatch-marked areas presented in Table 6.

Minimum detection limit and FP/FN

Throughout the following section, minimum detection limit and FP/FN results are used to help determine the accuracy and validity of the new, field-based tests presented here. Therefore, it is important to understand the inherent differences between these two terms.

First, ‘detection limits’ refers to the lowest concentration of a substance (in this case, *E. coli* or H₂S-producing bacteria) that can be distinguished from the absence of that substance. Therefore, ideally, a microbiological test would have a low minimum detection limit (i.e. the test is said to

Table 5 | Calculations for error – exact match (EC-Kit only)

		Quanti-Tray		
		Conformity/low	Intermediate	High/very high
Water source type + EC-Kit	Conformity/low			
	Intermediate			
	High/very high			

Table 6 | Calculations for error for single, new, field-based tests and testing combinations

		Quanti-Tray		
		Conformity/low	Intermediate	High/very high
Water source type + EC-Kit	Conformity/low			
	Intermediate			
	High/very high			

be 'sensitive') so that it could detect the presence of a substance, even at low concentrations. Second, FP/FN represents the percentage of sample results that were incorrect. More specifically, FPs represent results that were positive by the new, field-based test but which did not contain any of the looked-for substance. Conversely, FNs are results that were negative by the new, field-based test, but which contained the looked-for substance.

Detection limits and FP/FN results are both necessary in order to understand the test results given by the new, field-based microbiological tests. All in all, these comparative, statistical tools measure different things: FP/FN results tell us how good a given test is at quantifying the results; whereas, the minimum detection limit tells us how good the test is at receiving a (low) signal.

Colilert results

The frequency distribution table for the Colilert test results compared to Quanti-Tray are presented in Table 7. The corresponding TR, FP and FN values are presented in Table 8 for Capiz samples.

The Colilert test yielded relatively accurate results (75%). However, it is of concern that the FN (19%) was much higher than the FP (6%). This may be because the detection limit for the Colilert test is 10 colony forming units (CFU) per 100 mL, or 1 CFU per 10 mL of sample. For the purposes of distinguishing between unimproved and improved water sources, these false results do not err on the side of caution. Table 9 presents the error and proportional reduction in error values for the Colilert test results. It is interesting to note that the addition of the Colilert test greatly improves the initial predictions based on UN-designated improved/unimproved categories alone. In fact, for unimproved sources, it decreased the error by 25%, and for improved sources, it decreased the error by 58%, resulting in a more accurate description of the water

Table 7 | Frequency distribution table for Colilert, Capiz samples

		Quanti-Tray	
		Presence	Absence
Colilert test	Presence	242	32
	Absence	101	146

Table 8 | TR, FP and FN results for Colilert test, Capiz samples

	True results (%)	False positives (%)	False negatives (%)
Colilert test	75	6	19

source. However, it should be noted that the error following the addition of the Colilert test is still at 12% for unimproved and 27% for improved sources.

Petrifilm results

The frequency distribution table for the Petrifilm test results compared to Quanti-Tray are presented in Table 10. The corresponding TR, FP and FN values are presented in Table 11 for Capiz samples.

The Petrifilm test yielded highly accurate results (88%). The FNs had Petrifilm indicating a higher risk level category than Quanti-Tray. While ideally one would hope for perfect congruence of the two test methods, it is preferable to have over-reporting of risk (false positives) than under-reporting of risk (false negatives). Table 12 presents the error and proportional reduction in error values for the Petrifilm test results.

Table 9 | Error and proportional reduction in error for Colilert test, Capiz samples

Test	Unimproved sources Initial error = 15%			Improved sources Initial error = 64%		
	Error	λ	n	Error	λ	N
Colilert	12%	25%	521	27%	58%	521

Table 10 | Frequency distribution table for Petrifilm test, Capiz samples

		Quanti-Tray	
		Presence	Absence
Petrifilm test	Presence	353	19
	Absence	43	106

Table 11 | TR, FP and FN results for Petrifilm test

	True results (%)	False positives (%)	False negatives (%)
Petrifilm test	88	4	8

Table 12 | Error and proportional reduction in error for Petrifilm, Capiz samples

Test	Unimproved sources Initial error = 15%			Improved sources Initial error = 64%		
	Error	λ	n	Error	λ	n
Petrifilm	37%	-138%	521	39%	39%	521

For unimproved sources, the addition of Petrifilm did not reduce our error, but in fact increased it ($\lambda = -138\%$). Therefore, as a single test for unimproved sources, Petrifilm yields a much less accurate prediction than predicting that all unimproved sources are contaminated. For improved sources, the addition of Petrifilm significantly reduced the error by 39%, with an error of 39%.

H₂S test results

The frequency distribution table for the H₂S bacteria test results compared to Quanti-Tray test results are presented in Table 13. The corresponding TR, FP and FN values are presented in Table 14 for samples collected in Capiz and Cambridge.

The H₂S medium was used to test 163 water samples in Capiz Province from different sources (springs, protected

Table 13 | Frequency distribution tables for H₂S bacteria tests

10-mL H₂S test		Quanti-Tray	
		Presence	Absence
10-mL H₂S test	Presence	112	19
	Absence	22	50
20-mL H₂S test		Quanti-Tray	
		Presence	Absence
20-mL H₂S test	Presence	122	20
	Absence	12	49
HACH Pathoscreen		Quanti-Tray	
		Presence	Absence
HACH Pathoscreen	Presence	110	19
	Absence	24	50
100-mL H₂S test		Quanti-Tray	
		Presence	Absence
100-mL H₂S test	Presence	126	32
	Absence	8	36

Table 14 | TR, FP and FN Results for H₂S bacteria tests

		True results (%)	False positives (%)	False negatives (%)
Laboratory-made reagents	10-mL H₂S test (n = 203)	80	9	11
	20-mL H₂S test (n = 203)	84	10	6
	100-mL H₂S test (n = 203)	80	16	4
Manufactured and purchased	HACH test (n = 203)	79	9	12

and unprotected open dug wells, rainwater, shallow and deep bore wells and chlorinated and un-chlorinated household taps) and to test 40 samples in Cambridge, Massachusetts: 38 from the Charles River, and 2 de-ionized water samples.

When comparing the H₂S laboratory-made reagents, surprisingly, the 20-mL test gave slightly more TR (84%) than the 100-mL (80%) and 10-mL tests (80%). As would be expected, the percentage of FP results was highest for the 100-mL test (16%) and lowest for the 10-mL test (9%); whereas the percentage of FNs was highest for the 10-mL test (11%) and lowest for the 100-mL test (4%).

In general, it was noted that as the sample volume of the H₂S test increased, FP increased (in fact, becoming overly sensitive, with 16% FP results) and FN decreased (becoming sensitive).

The 20-mL HACH PathoScreen test had results that were very similar to the 10-mL H₂S test, although it still proved to be the least accurate of all the H₂S tests: it had the lowest percentage of true results (79%) and although its percentage of FPs was low (9%), it had the highest percentage of FNs (12%).

The high percentage of FP results (16%) for the 100-mL test is probably due to the H₂S bacteria test detecting H₂S that may not come from faecal bacteria. In groundwater in particular, there is the strong possibility of H₂S being present due to natural hydrogeological sources and to anthropogenic impacts other than faecal contamination, both of which would lead to FP results (Sobsey & Pfaender 2002). This phenomenon is important in this study since most drinking water samples (136 samples) tested using

the H₂S test, were groundwater collected from wells and spring sources. Furthermore, it has been shown that the H₂S test detects bacteria other than coliforms that are associated with faecal contamination, such as *Clostridium perfringens*, which is one of the most resistant indicators of faecal contamination. Therefore, it is possible that the H₂S test can yield a positive result even if no coliforms are present (Sobsey & Pfaender 2002).

Of great concern with microbiological tests in general is the potential for FNs, in other words not detecting faecal contamination when it is present. The percentage of FNs was relatively low for the 10-mL sample (11%), but was reduced by about half in the 100-mL sample (4%). The higher percentage of FPs versus FNs in the 20- and 100-mL tests is favourable because it errs on the side of caution. For the 10-mL H₂S test and HACH tests, the percentage of FNs was higher than the percentage of FPs.

Table 15 presents the error and proportional reduction in error values for the H₂S bacteria test for Capiz samples only, since the water source used in Cambridge was not a drinking water source and therefore could not be deemed 'unimproved' or 'improved'.

It is interesting to note that errors for the H₂S test were greater for improved sources than for unimproved sources. For unimproved sources, the addition of the 10-, 20- and 100-mL H₂S test did not change the error ($\lambda = 0\%$); however, the addition of the HACH test *increased* the error by 133%. Therefore, as a single test for unimproved sources, the laboratory-made H₂S test is no better than simply predicting that all unimproved sources are contaminated. For improved sources, the addition of all H₂S tests (laboratory made and HACH test) reduced the error on average by 53%, with an average error of 24%.

Easygel test results

The frequency distribution table for the Easygel test results compared to Quanti-Tray test results is presented in Table 16. The corresponding TR, FP and FN values are presented in Table 17 for Capiz and Cambridge samples combined.

The Easygel test was used to test 41 water samples in Capiz Province from different sources (springs, protected and unprotected open dug wells, deep bore wells and

Table 15 | Error and proportional reduction in error for H₂S bacteria tests, Capiz samples only

Test	Unimproved sources			Improved sources		
	Error (%)	λ	n ¹	Error (%)	λ	n ²
10-mL H ₂ S test	9.1	0.0	33	24.6	51.5	130
20-mL H ₂ S test	9.1	0.0	33	20.0	60.6	130
100-mL H ₂ S test	9.1	0.0	33	28.7	43.9	129
HACH test	21.2	-133	33	23.1	54.6	130

¹ Sample size for unimproved sources tested for given H₂S test.

² Sample size for improved sources tested for given H₂S test.

chlorinated and un-chlorinated household taps) and to test 40 samples in Cambridge, Massachusetts: 38 from the Charles River, and 2 de-ionized water samples.

The Easygel test had a relatively high percentage of TRs (81%), few FPs and a high proportion of FNs. This means that the Easygel test is a particularly good indicator of the presence of *E. coli* contamination, but not of the absence of contamination.

Table 18 presents the error and proportional reduction in error for the Easygel test for Capiz samples only, since the water source used in Cambridge was not a drinking water source and could therefore not be deemed an 'unimproved' or 'improved' water source.

For unimproved sources, the addition of Easygel did not reduce our error, but in fact increased it ($\lambda = -100\%$). Therefore, as a single test for unimproved sources, the Easygel test yields a less accurate prediction than predicting that all unimproved sources are contaminated. For improved sources, the addition of Easygel reduced our error by 51.5%, with an error of 24.6%. Fisher's exact test on the

Table 16 | Frequency distribution table for Easygel test

		Quanti-Tray	
		Presence	Absence
Easygel	Presence	49	1
	Absence	14	17

Table 17 | TR, FP and FN results for Easygel test

	True results	False positives (%)	False negatives (%)
Easygel (n = 83)	81	1	17

contingency table for unimproved and improved sources in Capiz showed that these results are not statistically significant, due to their small sample size.

The 3×3 frequency distribution table for the Easygel test compared to Quanti-Tray test results is presented in Table 19. This table presents the Easygel and Quanti-Tray test results in three categories: the WHO Risk Levels (Conformity/Low, Intermediate and High/Very High) for Capiz and Cambridge samples.

The majority of samples (51) were identically classified by the Easygel test and Quanti-Tray. The true results percentage (i.e. results that lie in the same WHO Risk Level for the Easygel test and Quanti-Tray) for this 3×3 frequency distribution table is 64%. However, what is most important here is that the WHO Risk Level for a given sample, obtained by the Easygel test, corresponds to the same or a lower-risk WHO Risk Level (shaded region in Table 19). In this light, the true results percentage (i.e. results that lie in the same or higher WHO Risk Level for the Easygel test than Quanti-Tray) is 75%. Again, such misclassifications err on the side of caution as it can result in the rejection of water that may be safe to drink as opposed to acceptance of water that is unsafe.

Table 18 | Error and proportional reduction in error for Easygel test, Capiz samples only

Test	Unimproved sources			Improved sources		
	Error	λ	n^1	Error	λ	n^2
Easygel	28.6%	-100%	14	24.6%	51.5%	28

¹ Sample size for unimproved sources.

² Sample size for improved sources.

Table 19 | 3×3 frequency distribution table for Easygel test, Capiz and Cambridge samples

		Quanti-Tray		
		Low/ conformity	Intermediate	High/very high
Easygel	Low/ conformity ¹	22	9	0
	Intermediate ¹	3	5	11
	High/very high ¹	0	7	24

¹ The WHO risk levels were determined based on the sample volume used in the Easygel test (5 mL) compared to the actual risk levels based on a 100 mL sample.

Low/Conformity: 0 CFU/5 mL, Intermediate: 1 to 4 CFU/5 mL, High/Very High: >5 CFU/5 mL.

Test combinations

The accuracy of different testing combinations of presence/absence test (Colilert and H₂S bacteria tests) and enumerative test (Petrifilm and Easygel) was analysed. These combinations were compared statistically to Quanti-Tray using the 3×3 frequency distribution table and again looking at the error and proportional reduction in error. The test combinations are presented in Table 20.

Test results and corresponding WHO Risk Levels were set up for the different test combinations and are presented here for EC-Kit (Colilert + Petrifilm) (Table 21), H₂S test + Petrifilm (Table 22), Colilert + Easygel (Table 23) and H₂S test + Easygel (Table 24). The corresponding WHO Risk Levels for Easygel were for a sample volume of 5 mL.

It should be noted that the associated WHO Risk Levels should not be taken as 'absolutes' but, rather, as an initial benchmark with which to compare test combinations to Quanti-Tray results.

The 3×3 frequency distribution table for the EC-Kit test results is presented in Table 25. The 3×3 contingency tables for the testing kit combinations compared Quanti-Tray to improved sources and unimproved sources separately. The corresponding error and proportional reduction in error (λ) are presented in Table 26. This table presents values for Capiz samples only, since the water source used in Cambridge was not a drinking water source and could therefore not be deemed an 'unimproved' or 'improved' water source.

Table 20 | Testing combinations of new, field-based tests

Test combinations

Colilert + Petrifilm (EC-Kit)
10-mL H ₂ S test + Petrifilm
20-mL H ₂ S test + Petrifilm
100-mL H ₂ S test + Petrifilm
20-mL HACH test + Petrifilm
Colilert + Easygel
10-mL H ₂ S test + Easygel
20-mL H ₂ S test + Easygel
100-mL H ₂ S test + Easygel
20-mL HACH test + Easygel

Table 21 | WHO risk levels and corresponding EC-Kit test results. Adapted from WHO (1997), replacing 'thermotolerant bacteria' with '*E. coli*'

WHO risk level	<i>E. coli</i> in sample (CFU/100 mL)	Colilert <i>E. coli</i> result	Petrifilm <i>E. coli</i> result
Conformity	<1	Clear	0
Low	1–10	Clear	0
Intermediate	10–100	Blue fluorescence	0
High	100–1000	Blue fluorescence	1–10
Very high	>1000	Blue fluorescence	>10

Table 22 | WHO risk levels and corresponding H₂S test + Petrifilm test results

WHO risk level	H ₂ S test result	Petrifilm result (CFU/mL)
Conformity	Yellow	0
Low	Yellow	0
Intermediate	Black	0
High	Black	1–10
Very high	Black	>10

Table 23 | WHO risk levels and corresponding Colilert and Easygel test results

WHO risk level	Colilert result	Easygel result (CFU/5 mL)
Conformity	Clear	0
Low	Clear	0
Intermediate	Blue fluorescence	0–4
High	Blue fluorescence	5–50
Very high	Blue fluorescence	>50

Table 24 | WHO risk levels and corresponding H₂S test and Easygel test results

WHO risk level	H ₂ S test result	Easygel (CFU/5 mL)
Conformity	Yellow	0
Low	Yellow	0
Intermediate	Black	0–4
High	Black	5–50
Very high	Black	>50

As previously mentioned, the calculations for error for EC-Kit were done in two ways. The 'exact match' method is useful information for the verification of EC-Kit, and the results indicate that the EC-Kit has a 25–29% error for exactly matching the Quanti-Tray results. However, as

Table 25 | 3 × 3 frequency distribution table for EC-Kit tests, Capiz samples (n = 521 samples)

		Quanti-Tray		
		Low/conformity	Intermediate	High/very high
EC-Kit	Low/conformity ¹	230	13	4
	Intermediate ¹	76	34	15
	High/very high ¹	13	30	106

explained above, these calculations are not as useful for water quality testing because we are not so much concerned with *exactly* matching Quanti-Tray so much as providing information on water sources unsuitable for consumption. The second method (which includes overestimates of risk level, Table 6) is a preferable statistical analysis approach. This is because an overestimate of the risk level still yields useful information for someone interested in knowing their water quality. In other words, it errs on the

Table 26 | Error, proportional reduction in error (λ) and sample number, n for unimproved and improved samples collected in Capiz and compared to Quanti-Tray

	Unimproved sources			Improved sources		
	Error (%)	λ	n ¹	Error (%)	λ	n ²
EC-Kit (Colilert + Petrifilm (EXACT MATCH))	25	–63	521	29	54	521
EC-Kit (Colilert + Petrifilm)	6	63	521	6	60	521
10-mL H ₂ S test + Petrifilm	9.1	82	33	3.5	93	114
20-mL H ₂ S test + Petrifilm	12	–33	33	2.4	95	126
100-mL H ₂ S test + Petrifilm	6.1	33	33	1.6	97	125
20-mL HACH test + Petrifilm	15	–67	33	1.6	97	125
Colilert + Easygel	0.0	100	13	0.0	100	28
10-mL H ₂ S test + Easygel	0.0	100	4	0.0	100	18
20-mL H ₂ S test + Easygel	0.0	100	4	0.0	100	19
100-mL H ₂ S test + Easygel	0.0	100	3	0.0	100	19
20-mL HACH test + Easygel	0.0	100	3	0.0	100	22

¹ Sample size for unimproved sources.² Sample size for improved sources.

conservative side of over-prediction rather than under-prediction. Given these facts, the addition of both tests in the form of an EC-Kit would improve predictions by 60–63% for both improved and unimproved water sources.

In general, for unimproved and improved sources, the combination of tests yielded better prediction of faecal contamination than single tests, with the exception of 20-mL H₂S test + Petrifilm ($\lambda = -33\%$ for unimproved sources) and 20-mL HACH test + Petrifilm ($\lambda = -67\%$ for unimproved sources). In other words, the 20-mL H₂S test alone or the assumption of contamination based on source type without any testing are better predictors than the 20-mL H₂S test + Petrifilm or 20-mL HACH test + Petrifilm combinations.

It is interesting to note that all combinations that included Easygel reduced the error by 100%, such that error = 0%. This proportional reduction in error is much larger than the proportional reduction in error obtained for Easygel alone (-100% for unimproved and 51.5% for improved). This large difference in λ can be attributed to the properties of the P/A tests that were combined with Easygel. As a matter of fact, the Easygel test yielded few FP results (1%) and many FN results (17%). On the other hand, the H₂S tests that were combined with Easygel had many FP results (9 to 16%) and few FN results (4 to 11%). This could mean that the two tests effectively complement one another, such that the λ value of the combined tests is significantly greater than the λ value of a single test.

Finally, it must be mentioned that the sample size for these Easygel combinations was particularly small, especially for unimproved sources (3 to 4 samples).

Cost analysis

The cost summaries presented here are only for the new, field-based microbiological tests: EC-Kit (Colilert and Petrifilm), H₂S bacteria test and Easygel. The cost of the Standard Methods tests (Quanti-Tray and membrane filtration) were not included in this paper because, throughout this project, these tests were used for verification purposes and as the Standard Methods against which to test the field-based methods. More specifically, not only are these tests expensive (Quanti-Tray tests ranged from \$6 in USA to \$21 in Philippines per sample) but they also require the use of other expensive equipment (sealer and incubator).

EC-Kit

Currently, EC-Kits are being assembled and disseminated by Susan Murcott, Senior Lecturer in the Civil and Environmental Department at MIT, as part of a research and mapping project. These kits are sold at cost.

At this time, four models (Model A through D) are available. Every model contains sterile sample bags, individually wrapped sterile pipettes, a portable UV lamp with 4 AA batteries, an insulated cooler bag, waist-belt incubator, ice pack and laminated instructions. The price of each kit model are given in Table 27 including the price per test (without the rest of the kit contents) of \$1.50/Colilert test and \$1.25/Petrifilm test. Also, these costs do not include the cost of domestic US postage, which can range from \$5 to \$20 depending on the kit size and speed of delivery; or the cost of international postage.

H₂S test

The H₂S test, or the H₂S paper strip test, requires the use of readily available laboratory reagents, distilled or de-ionized water, paper towels or toilet paper and vials or sterile sampling bags. The cost of the paper strip (non-toxic absorbing paper) is included in Table 28, but the cost of the vials or sampling bags is not included.

The cost for the H₂S paper strip test (laboratory-made M2 reagent) was calculated based on the price of reagents required to make 2.5 L of H₂S reagent solution (5000 tests for the 10-mL H₂S test, 2500 for the 20-mL H₂S test and 1000 for the 100-mL H₂S test). The price and units for all reagents were taken from Sigma Aldrich (www.sigmaaldrich.com), except for sodium thiosulfate, for which the price and units were obtained from VWR (www.vwr.com). Prices were obtained for orders based in the United States and in the Philippines: \$344 and \$830, respectively. It is important to note that the price of reagents in the Philippines is almost 2.5 times higher than the price of the same reagents in the United State (The prices shown in Table 28 assume purchase in the US).

The HACH P/A media is available directly from the HACH website. Typically, one pouch (or 'powder pillow') is used as the test reagent for a 20-mL sample volume. A

Table 27 | Contents and cost of EC-Kit Model A, B, C and D

	Kit contents	Total cost (\$)	Number of tests	Cost (\$)/test Complete Kit	Cost (\$)/test Test reagents only
Model A (C-10)	<ul style="list-style-type: none"> • 10 Colilert tests 	32.00	10	3.20	1.50
Model B (CP-25)	<ul style="list-style-type: none"> • 25 Colilert tests • 25 3M Petrifilm (1 pack) • Incubator belt • 2 ice packs • 10 cardboard squares • 20 rubber bands 	104.00	25	4.16	2.75
Model C (CP-50)	<ul style="list-style-type: none"> • 50 Colilert tests • 50 3M Petrifilm (1 pack) • Incubator belt • 2 ice packs • 20 cardboard squares • 40 rubber bands 	187.00	50	3.74	2.75
Model D (CP-100)	<ul style="list-style-type: none"> • 100 Colilert tests • 100 3M Petrifilm (1 pack) • Incubator belt • 2 ice packs • 30 cardboard squares • 60 rubber bands 	349.00	100	3.49	2.75

pack of 50 powder pillows is \$29.39, or approximately 59¢ per test.

Table 28 presents the average cost per test for the three different laboratory-made H₂S tests and HACH tests, from a 2.5 L reagent solution for just the test reagents themselves, not including the sample vial or sampling bag.

Easygel

The Easygel test requires a specially pre-treated Petri dish and the Easygel media. These are sold as a test kit (one kit is comprised of one medium bottle and one treated Petri dish) from Micrology Laboratories (www.micrologylabs.com) and are available in sets of ten tests for \$21.25/set if one to nine sets are purchased and for \$16.25/set if more than ten sets are purchased. This means that individual tests range from \$1.63 to \$2.13.

Cost comparison

Table 29 compares the cost of each microbiological test, assuming purchase in the US. The H₂S tests (10-, 20-, 100-mL and HACH) were by far the least expensive of the microbiological

tests (less than 60¢ each), excluding the initial cost of glass vials, 100-mL sterile sampling bags and paper strips. The Easygel tests, however, the H₂S, the Easygel and Model A of the EC-Kit are for individual tests.

The H₂S (20 ml) + the Easygel is a two – test system, as it the EC-Kit, Models B, C and D. The EC-Kit Models B, C or D is \$1.00 more expensive per test set than the H₂S (20 ml) + the Easygel test set.

Table 28 | Average cost per test for different H₂S test sample volumes, from a 2.5 L reagent solution

	Laboratory-made H₂S test sample volume			HACH test 20-mL
	10 mL	20 mL	100 mL	
Reagent volume/test (mL)	0.5	1.0	2.5	n/a
No. of samples tested	5000	2500	1000	n/a
United States – Average cost/test¹ (\$)	0.07	0.14	0.35	0.59
Philippines – Average cost/test¹ (\$)	0.17	0.33	0.83	n/a

n/a: not applicable.

¹ The average cost per test was calculated based on the cost of laboratory reagents for 2.5 L of solution, adding the cost of the paper towels. The cost of vials/bottles and sampling bags was not included in the average cost/test.

Table 29 | Cost/test of H₂S test, Easygel and EC-Kit in the USA

Test	H ₂ S test			Easygel			H ₂ S test (20 ml) + Easygel	H ₂ S test (100 ml) + Petrifilm	EC-Kit: Colilert + Petrifilm
	10 mL ¹	20 mL ¹	100 mL ¹	HACH	1-9 sets	10+ sets			
Cost/test (\$)	0.07	0.14	0.35	0.59	2.13	1.63	1.77	1.60	2.75

¹ The cost data presented in this table for the H₂S test reflects cost incurred in the United States in order to provide an adequate comparison with the Easygel and EC-Kit

Other considerations

Finally, the figures cited here represent an approximate cost of each test. It is important to consider that prices differ greatly from country to country, and that costs of reagents and laboratory supplies are usually more expensive in developing countries. Also, one may need to consider freight/transportation costs associated with shipping the reagents to remote locations worldwide.

RECOMMENDATIONS

Table 30 provides a summary of the TR, FP and FN values for the individual, field-based tests.

Given the statistical analyses from each of the individual microbiological tests, one individual test cannot be recommended as an accurate and/or suitable microbiological test for drinking water. In fact, although Petrifilm had 88% true results, it must be noted that Petrifilm has a high detection limit (i.e. it can only detect microbiological contamination in samples that have a concentration of 100 CFU/100 mL, and are above the WHO Intermediate Risk Level); therefore it is unsuitable for water sources with low *E. coli* concentrations. The 20-mL H₂S test also provided

reasonably good results (with 88% true results and 4% and 8% of false positives and false negatives, respectively).

However, once assumptions are made regarding the water source type (e.g. all doubtful sources are unimproved), then from the calculations of error and proportional reduction in error for unimproved and improved water sources, it is possible to improve predictions with the use of a single test.

The statistical analyses also showed that the EC-Kit performs very well in predicting water contamination (6% error). The 100-mL H₂S test in conjunction with Petrifilm also yielded highly promising results (6.1% error for unimproved sources and 1.6% error for improved sources).

However, the most accurate testing combination was the H₂S tests (10-mL, 20-mL, 100-mL and 20-mL HACH) combined with Easygel. Although these combinations all yielded the same error and proportional reduction in error (0% and 100%, respectively) the 20-mL H₂S test + Easygel combination was chosen as the best option based on the accuracy of the individual 20-mL H₂S test. However, it must be noted that the both the 20-mL H₂S tests and Easygel were performed on a very small sample size (23 samples only: 4 unimproved sources and 19 improved sources), therefore it is recommended that the 20-mL H₂S tests and Easygel combination be verified in future studies, on a much larger scale. It is also recommended that due to the limitations present in the detection limits for Quanti-Tray and Petrifilm, future projects verifying the EC-Kit should use Quanti-Tray/2000.

Table 30 | Summary of TR, FP and FN values for individual tests

	True results (%)	False positives (%)	False negatives (%)	n
Colilert	75	6	19	521
Petrifilm	88	4	8	521
10-mL H ₂ S test	80	9	11	203
20-mL H ₂ S test	84	10	6	203
100-mL H ₂ S test	80	16	4	203
20-mL HACH test	79	9	12	203
Easygel	81	1	17	83

CONCLUSIONS

The primary objective of this study was to verify and assess the suitability of four, low-cost, microbiological field-based tests for drinking water quality testing. More specifically, the study examined the 10-mL pre-dispensed Colilert test, the Petrifilm test, the laboratory-made P/A

H₂S test (for 10-, 20- and 100-mL sample volume), the HACH PathoScreen P/A H₂S test (20-mL sample volume) and the Easygel test. The different tests were verified and compared for accuracy. Accuracy was measured by comparing the field test results to the results obtained using a Standard Methods test (Quanti-Tray).

The drinking water samples used in this study were collected in different municipalities throughout Capiz Province, Philippines, in January 2010, and from the Charles River in Cambridge, Massachusetts, in April 2010. In total, 521 samples were tested using the Colilert and Petrifilm tests; 203 samples were tested using the H₂S tests; and 83 samples were tested using the Easygel test.

The tests were evaluated as single tests, and as a combination of tests (i.e. Colilert and Petrifilm, H₂S test and Petrifilm, H₂S test and Easygel) to determine the best testing combination.

Based on the statistical analyses conducted, the results from this study do not support the use of one single microbiological test as a suitable method for determining drinking water quality accurately. That said, the single 20-mL laboratory-made H₂S test yielded the best results with 88% true results, 4% false positives and 8% false negatives.

Test combinations proved to be promising alternatives to single tests for accurate results. In fact, combining tests such as the Colilert and Petrifilm (EC-Kit) yielded very good results, with an overall error of 6% (compared to 25% and 12% for the single tests, Colilert and Petrifilm, respectively). And although statistical analyses comparing the EC-Kit with Quanti-Tray revealed that the results from both tests did not exactly match, the EC-Kit's low overall error of 6% means that it is indeed very useful in assessing at-risk water sources.

The 100-mL H₂S test and Petrifilm test combination also yielded highly promising results (6.1% error for unimproved sources and 1.6% error for improved sources), but the most accurate testing combination was determined to be the 20-mL H₂S test and Easygel combination, since it yielded an error of 0% and since the 20-mL H₂S test was the most accurate single test evaluated here.

These two testing combinations were also inexpensive (Table 29). The 100-mL H₂S test and Petrifilm cost \$1.60/

test (\$0.35 for the 100-mL H₂S test reagent only and \$1.25 for Petrifilm, if purchased in the United States), and the 20-mL H₂S test and Easygel cost \$1.77/test (\$0.14 for the 20-mL H₂S test reagent only and \$1.63 for Easygel, if purchased in the United States). These prices are less expensive than the EC-Kit two-test combination, which costs \$2.75/test (\$1.75 for Colilert and \$1.25 for Petrifilm Colilert if only the tests themselves are considered and if purchased in the United States).

Although the 20-mL H₂S test and Easygel combination was the most promising testing combination, it cannot yet be recommended for extensive use in microbiological testing of drinking water in developing countries since the sample size for this particular combination was very small (23 samples). Therefore, it is recommended that the 20-mL H₂S tests and Easygel combination be verified in future studies, on a much larger scale. It is also recommended that due to the limitations present in the detection limits for Quanti-Tray and Petrifilm, future projects verifying the EC-Kit should use Quanti-Tray/2000 as the standard for comparison.

REFERENCES

- CIA 2009 *CIA The World Factbook*. Retrieved February 20, 2010, from Central Intelligence Agency: <https://www.cia.gov/library/publications/the-world-factbook/geos/rp.html>.
- Gerba, C. P. 2000 Indicator Microorganisms. In: *Environmental Microbiology* (ed. R. Maier, I. Pepper & C. Gerba). Academic Press, San Diego, pp. 491–503.
- IDEXX 2010 *IDEXX February 2010 Newsletter*. Retrieved February 20, 2010, from IDEXX: www.idexx.com/view/xhtml/en_us/water/newsletter/201002.jsf.
- Manja, K., Maurya, M. & Rao, K. 1982 A simple field test for the detection of faecal pollution in drinking water. *Bull. WHO* **60**, 797–801.
- Micrology Laboratories 2009 *Coliscan Easygel*. Retrieved February 21, 2010, from Micrology Laboratories: http://micrologylabs.mennonite.net/Home/Our_Methods/Coliscan_Media/Coliscan_Easygel.
- Micrology Laboratories 2008 *Coliscan Easygel Guide*, Goshen, IN.
- NSO 2002 *Press Release – Census – 2002*. Retrieved February 27, 2010, from National Statistics Office of the Philippines: <http://www.census.gov.ph/data/pressrelease/2002/pr0276tx.html>.
- Pillai, J., Mathew, K., Gibbs, R. & Ho, G. 1999 H₂S paper strip method – A bacteriological test for faecal coliforms in

- drinking water at various temperatures. *Water Sci. Tech.* **40** (2), 85–90.
- Sobsey, M. & Pfaender, F. 2002 Evaluation of the H₂S method for detection of fecal contamination of drinking water. WHO, Geneva.
- United Nations 2000 UN Millennium Development Goal 7, Target 7C. Retrieved October 11, 2010. <http://www.un.org/millenniumgoals/environ.shtml>.
- Venkobachar, C., Kumar, D., Talreja, K., Kumar, A. & Iyengar, L. 1994 Assessment of bacteriological water quality using a modified H₂S strip test. *J. Water Supply Res. Technol. – Aqua* **43** (6), 311–314.
- WHO 1997 *Guidelines for Drinking-Water Quality*, vol. 3, Surveillance and control of community supplies, Geneva, Switzerland.
- WHO/UNICEF 2010 *Progress on Sanitation and Drinking-Water 2010 Update*. WHO, Geneva.
- WHO/UNICEF Joint Monitoring Programme 2005 *Water for Life: Making it Happen*. WHO, Geneva. Retrieved March 21, 2011. http://www.unicef.org/media/files/JMP_05_text.pdf.

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