STUDY OF FOUR NEW, FIELD-BASED, MICROBIOLOGICAL TESTS: VERIFICATION OF THE HYDROGEN SULFIDE (H_2S), EASYGEL®, COLILERT AND PETRIFILM™ TESTS

By

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ABSTRACT

Currently, the U.N. defines water sources as "improved" (e.g. public taps, protected dug wells and springs, rainwater collection) and "unimproved" (e.g. surface waters, unprotected dug well and spring, and vended water). Although these water quality indicators are easy to measure, they do not reflect the actual quality of the drinking water source. A more accurate method of determining drinking water quality is to perform laboratory drinking water quality tests. Laboratory testing is especially difficult in developing countries where funds, technology, laboratory facilities, and trained laboratory personnel are lacking. Fortunately, over the last 30 years, scientists, researchers and inventors have developed a series of low-cost, microbiological, field-based tests. These include the Presence/Absence (P/A) hydrogen sulfide (H_2S) test, the enumerative Easygel® test, the 10-mL P/A Colilert test and enumerative Petrifilm™ test. However, the accuracy of these tests has never verified or established.

The objective of this thesis is fourfold: (1) to verify the accuracy of the four field-based tests: the H_2S tests (laboratory-made reagent for 10-, 20- and 100-mL sample volume, and industry-made HACH PathoScreen™ reagent), Easygel®, Colilert and Petrifilm™, by comparing them to two Standard Methods tests: Quanti-Tray® and membrane filtration; and to assess these tests based on two other factors: cost and practicality/ease of use; (2) to assess the suitability of the H_2S-producing bacteria as an indicator of fecal contamination; (3) to provide recommendations for the use of a single P/A test and a single enumerative test (Petrifilm™ or Easygel®) to be used on the field; and (4) to provide recommendations for a testing combination made up of one P/A test and one enumerative test.

The tests used in this study were conducted on water samples collected from Capiz Province, Philippines, and from the Charles River, Cambridge, MA.

The H_2S-producing bacteria was found to be a valid indicator of fecal contamination. However, further testing is recommended to ensure that the H_2S-producing bacteria meet all the WHO requirements for an ideal indicator of fecal contamination.

The study recommends the use of the 20-mL H_2S test and the Colilert test as a single P/A test for testing improved and unimproved water sources, respectively. The use of the Easygel® test as a single enumerative test is recommended for testing improved water sources, and the use of the other enumerative tests (Easygel® and Petrifilm™) is strongly discouraged for unimproved sources. The combination of the 20-mL H_2S test and Easygel® combination is recommended for field-based microbiological drinking water quality testing.

Thesis Advisor: Susan Murcott
Title: Senior Lecturer of Civil and Environmental Engineering

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List of Abbreviations

CFU	Colony Forming Units
DOH	Department of Health
DPH	Director of Public Health
E.coli	Escherichia coli
FC	Fecal Coliform
FN	False Negative
FP	False Positive
H2S	Hydrogen sulfide
m-ColiBlue24®	Medium for simultaneous detection of Total Coliform and *E.coli*
M.Eng.	Master of Engineering
MIT	Massachusetts Institute of Technology
mL	Milliliter
MPH	Master of Public Health
MPN	Most Probable Number
NGO	Non-Governmental Organization
NPV	Negative Predictive Value
NSCB	Philippines National Statistical Coordination Board
NSO	Philippines National Statistics Office
P/A	Presence/Absence
PHO	Provincial Health Office
PHP	Philippine Peso
PPV	Positive Predictive Value
®	Registered Trademark
RHU	Regional Health Unit
SI	Sanitary Inspector
TC	Total coliforms
TM	Trademark
TR	True Results
U.N.	United Nations
UNDP	United Nations Development Program
WHO	World Health Organization

1. Introduction

1.1. Water Quality Testing Around the World

1.1.1. Background

Water is essential for life: for sustenance, hygiene and livelihood generation. Safe drinking water is a fundamental human need and is one of the central pillars on which productive, healthy lives are built. One billion people (or 15% of the world population) today do not have access to this basic need (World Bank, 2009). The great majority of these are people in developing countries. Furthermore, 1.8 million people, most of whom are children under the age of five, die each year of waterborne illnesses related to unsafe drinking water (WHO/UNICEF, 2010). One of the first steps required to empower individuals and communities to take steps towards gaining access to this basic need, is being able to accurately determine the quality of their own water source. With access to water quality information, along with information about drinking water supply or treatment options and available financial and/or technical assistance, they are in a position to reduce waterborne illnesses. However, there is still a need to develop simple, affordable, and accessible water quality testing methods for people in developing areas to acquire this information.

Accurate data on the water quality of the sources is also a key factor to determine how to best use the available quantities of water for multiple uses. Adequate quantities of water used for productive purposes such as irrigation, are essential for livelihood generation. This is critical for development (Koppen et al, 2009). With better information on the quality of water, more informed decisions could be made on how to utilize the quantity of water available in an area. Thus, it is necessary for people to have access to the tools required to make decisions about water in order to improve their lives.

1.1.2. Drinking Water Contamination and U.N. Indicators of Water Quality

There are four broad categories of drinking water contamination: microbiological, chemical, physical/aesthetic, and radionuclides. However, this thesis will solely focus on microbiological[1] contamination of drinking water, which occurs when drinking water is

[1] In this thesis, the terms "microbiological" and "microbial" are used interchangeably.

contaminated at the source by human or animal faeces, or through inappropriate transportation, handling, or storage in vessels in the household.

The current U.N. indicator for the drinking water target is the "proportion of population using an improved drinking water source" (UNDG, 2003). The U.N. lists the following as being an "improved" drinking water source: household connection, public standpipe, borehole, protected dug well, protected spring, rainwater; and the following as being an "unimproved" drinking water sources: unprotected dug well, unprotected spring, cart with small tank/drum, surface waters and bottled water.

The main advantages of the current U.N. indicators are that they are easy to measure, usually through a simple, low-cost survey, and the data collected is easy to compile and compare to other data from other areas, and overtime. However, these indicators do not guarantee the safety or danger of a given water source and therefore are not true, reliable measures of "safe water". For example, groundwater collected from a protected dug well (improved source) may be contaminated from nearby improperly sited latrines or animal stalls; or from contaminants from near-well activities (washing, open defecation…) infiltrating the groundwater from a broken well apron or pump. Or, a piped connection might be contaminated if there are leaks in the water and sanitary distribution networks. Therefore, it is impossible to accurately assess the quality of a drinking water source without performing laboratory microbiological water quality analyses.

1.1.3. WHO Criteria for Water Quality

The WHO (2008) has established microbiological drinking water quality guidelines, summarized in Table 1-1. In addition to the WHO guidelines, it is important to know the health risk levels associated with the presence (and degree of presence) of a contaminant. Therefore, the WHO (1997) determined five Risk Levels (hereafter called the "WHO Risk Levels"): Conformity, Low, Intermediate, High and Very High, and their corresponding range of *Escherichia coli* (*E.coli*) concentration (Colony Forming Units (CFU)/100 mL or Most Probable Number (MPN)/100 mL)[1] are presented in Table 1-2.

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[1] CFU values represent a direct plate count of bacterial colonies. MPN values are statistical estimates that represent the "most probable" CFU count given a set of discrete presence/absence data points. In this study, CFU and MPN values were taken to be directly equivalent.

Although *E.coli* is one of the most widely used indicators of fecal pollution, thermotolerant coliform bacteria counts are an acceptable alternative (WHO, 2008).

Table 1-1. Guideline Values for Verification of Microbial Quality (WHO, 2008).

Organisms	Guideline value
All water directly intended for drinking *E.coli* or thermotolerant coliform bacteria	Must not be detectable in any 100-mL sample
Treated water entering the distribution system *E.coli* or thermotolerant coliform bacteria	Must not be detectable in any 100-mL sample
Treated water in the distribution system *E.coli* or thermotolerant coliform bacteria	Must not be detectable in any 100-mL sample

Table 1-2. WHO Risk Levels for *E.coli*
(Adapted from WHO (1997) replacing "thermotolerant bacteria" with "*E.coli*").

Risk Level	***E.coli* in sample (CFU or MPN/100 mL)**
Conformity	<1
Low	1-10
Intermediate	10-100
High	100-1000
Very High	>1000

1.2. Project Area

Most of the fieldwork described in this thesis took place during January 2010, in Capiz Province, Philippines. This section provides background information on the Philippines and Capiz Province.

1.2.1. Philippines

Philippines is an archipelago made up of more than 7,000 islands, located in Southeast Asia, between the Philippine, Celebes and South China Seas (Figure 1-1). It is a mountainous country with low-lying reaches along the coastline.

Figure 1-1. Map of the Philippines. (CIA, 2009).

It has a total land area of approximately 300,000 km^2 and an extensive coastline of over 36,000 km. It has a tropical marine climate with two monsoon seasons: the dry, northeast monsoon from November to April, and the wet, southwest monsoon from May to October (CIA, 2009).

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 infant mortality rate is 24 deaths per 1,000 live births and life expectancy is approximately 71 years. Despite the low infant mortality rate and long life expectancy, the country has a high risk of infectious diseases. In fact, the country has a high rate of food and water-related diseases such as bacterial diarrhea, hepatitis A, typhoid fever, dengue, malaria and Japanese encephalitis, which is worsened by the tropical marine climate (CIA, 2009). Furthermore, the increasing population density and increasing level of urbanization could potentially exacerbate these diseases if appropriate steps are not taken.

1.2.2. Capiz Province

Capiz Province is situated on the northeastern part of Panay Island, located in the Western Visayas (Figure 1-1). It has a land surface area of approximately 2,600 km^2 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 (NSCB, 2009). The province is divided into 17 areas: 16 municipalities and Roxas City (Figure 1-2).

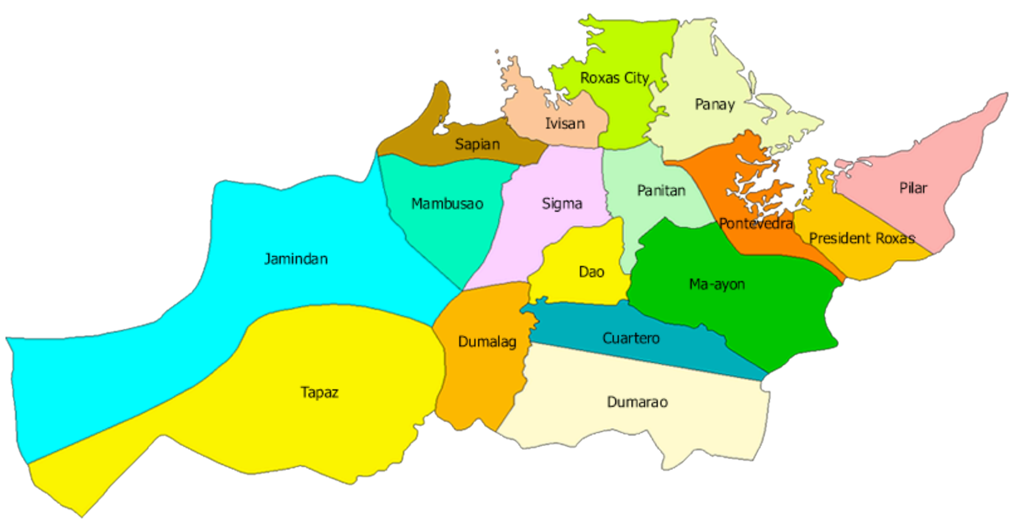

Figure 1-2. Map of Capiz Province and Municipalities (PhilRice Online, 2009).

The municipalities of Capiz are comprised of *barangays* (villages) and a *poblacion* (central district). Table 1-3 presents more detailed information on the different municipalities including population, number of *barangays*, income class and urbanization, as of August 2007. According to the Philippines National Statistical Coordination Board (NSCB, 2009), Capiz belongs to the 1st, and highest, income class, which means that the province's average annual revenues exceed PHP 350 M[1] (approximately US$ 7 M). Capiz's main economic activities are farming and fishing which use over 50% of the total land area of the province. Rice is the dominant agricultural crop, but sugar cane, coconuts, bananas and mango are also abundant. Seafood production is also common in Capiz, and the coastline is home to an increasing number of fishpond developments. Finally, the only urban area in Capiz is Roxas City, which is the center of trade and commerce. As a result, it is becoming increasingly industrialized and commercialized.

Table 1-3. Population, Income Class and Urbanization of Capiz Municipalities. (NSCB, 2009)

Municipality	Population	Number of *Barangays*	Income Range[1] (millions of PHP)	Urbanization[2]
Cuartero	25,306	22	20 – 30	Partially Urban
Dao	31,420	20	20 – 30	Partially Urban
Dumalag	29,221	19	20 – 30	Partially Urban
Dumarao	42,603	33	30 – 40	Partially Urban
Ivisan	25,882	15	20 – 30	Partially Urban
Jamindan	34,831	30	30 – 40	Partially Urban
Maayon	35,448	32	20 – 30	Partially Urban
Mambusao	37,498	26	20 – 30	Partially Urban
Panay	42,357	42	30 – 40	Partially Urban
Panitan	38,666	26	20 – 30	Partially Urban
Pilar	40,912	24	20 – 30	Partially Urban
Pontevedra	42,003	26	30 – 40	Partially Urban
President Roxas	28,459	22	20 – 30	Partially Urban
Roxas City	147,738	47	180 – 240	Urban
Sapian	23,552	10	20 – 30	Partially Urban
Sigma	28,709	21	20 – 30	Partially Urban
Tapaz	47,059	58	40 – 50	Partially Urban
Total	**418,755**	**473**	**670**[3]	

[1] Exchange rate is US$ 1 = PHP 45.00, dated May 11, 2010

[1] Income class is defined as the average annual income per municipality or city, and is listed in Philippines Peso (PHP) where
[2] Municipalities or cities are defined as "Partially Urban" if at least 1 constituent *barangay*, *poblacion* or central district meet the following criteria:
(1) Cities or provincial capitals have a population density greater than 1,000/km^2
(2) *Poblaciones* or central districts have a population density greater than 500/km^2
(3) *Poblaciones* or central districts not included in (1) and (2) regardless of the population size which have the following:
- street pattern or network of streets in either parallel or right angel orientation;
- at least six establishments (commercial, manufacturing, recreational and/or personal services);
- at least three of the following:
  a town hall, church or chapel with religious services at least once a month;
  a public plaza, park or cemetery;
  a market place, or building, where trading activities are carried on at least once a week;
  a public building, such as a school, hospital, kindergarten and health center or library.

(4) *Barangays* having a population of at least 1,000, which meet the conditions presented in (3) and where the occupation of the inhabitants is predominantly non-farming or fishing (NSCB, 2009).
[3] The total income was calculated by summing the median income range values
n/a: not applicable

1.3. Water Quality Testing in Capiz Province, Philippines

Until 2009, Capiz had never performed any drinking water quality testing on the various drinking water sources (wells, springs, surface water and piped supplies) used throughout the province, with the exception of those performed in the Roxas City municipal water treatment plant. The Provincial Health Office (PHO) of Capiz Province decided to undertake a water quality testing program throughout the province. The main PHO participants in this project included Dr. Jarvis Punsalan, MD, MPH, Director of Public Health (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 Sanitary Inspectors (SI's) at the provincial and municipal levels who were in charge of collecting the water samples and processing and interpreting one of the microbiological tests used.

During Fall 2008, Dr. Jarvis Punsalan received funding from the European Commission, the Philippines' government's Department of Health (DOH), and UNICEF to set up a water quality testing laboratory at Roxas Memorial Hospital, in Roxas City, which would test for drinking water 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: Quanti-Tray® and EC-Kit. Quanti-Tray® is an enzyme-substrate coliform test (Standard Methods 9223) based on Most Probable Number (MPN) and has been approved in more than 30 countries worldwide. The EC-Kit is a new portable microbiological testing kit comprised of two, easy-to-use tests: the 10-mL Presence/Absence (P/A) pre-dispensed

Colilert test and the enumerative test: 3M™ Petrifilm™ (Petrifilm™). The innovation of combining these two tests in the EC-Kit was the idea of Dr. Robert Metcalf, one of the original founders of the non-profit organization Solar Cookers International[1] and Professor of Microbiology at California State University at Sacramento. He introduced this method to Susan Murcott, in Kenya in 2005. She in turn developed and branded the EC-Kit, which combined all the items into a product, including the innovation of a waist belt incubator (section 5.2.3 provides more detail). Susan Murcott introduced the technology to the non-governmental organization (NGO) "A Single Drop", and introduced the director, Gemma Bulos, to Robert Metcalf, after which they brought the technology to the Philippines.

During 2009, Capiz's PHO purchased EC-Kits and Quanti-Tray® test reagents. An incubator, UV light and Quanti-Tray® sealer were also purchased in order to conduct the Quanti-Tray® tests. In May 2009, "A Single Drop" trained the Capiz PHO staff, municipal health officers and SI's on how to sample water sources, use the EC-Kit and interpret the sample test results. The Quanti-Tray® equipments 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. From October to December 2009, in collaboration with the MIT team, the PHO developed a water quality assessment survey designed to test 1,000 different water supplies from all 16 municipalities and Roxas City, which took place from December 2009 to March 2010. This would be the first-ever comprehensive drinking water quality testing in the province.

1.4. MIT Team

The MIT team was originally introduced to this project by Susan Murcott in September 2009. This project is a requirement for the Degree of Master of Engineering (M.Eng.) in Environment and Water Quality at MIT. The MIT team included Susan Murcott, as advisor and head of the MIT team, Patty Chuang, John Millspaugh, Molly Patrick and the author, Stephanie Trottier. Patty Chuang performed EC-Kit testing and verification with Quanti-Tray®; John Millspaugh constructed a Screening Model Optimization for Panay River Basin Planning and Management; Molly Patrick provided recommendations for at-risk water supplies in Capiz Province; the author verified the accuracy of new, field-based, microbiological tests: the hydrogen-sulfide (H_2S) test (H_2S test), Easygel®, and the 10-mL

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[1] www.solarcookers.org

P/A Colilert and Petrifilm™ (EC-Kit), and provided recommendations on these tests based on accuracy, cost, and practicality/ease of use.

1.5. Research Objectives

Although EC-Kits are now made up of the Colilert and Petrifilm™ tests, this effort to create and disseminate simple, low-cost microbiological testing products to be used in the field is not intended to be a one-time effort, but rather to evolve as new research improves on existing methods and as technologies emerge.

In addition to the EC-Kit tests (Colilert and Petrifilm™), two new tests were evaluated in this thesis: the P/A hydrogen sulfide bacteria test (H_2S test) and the enumerative Coliscan® Plus Easygel® (Easygel®) test. The H_2S tests used in the study included a laboratory-made reagent for different sample volumes (10 mL, 20 mL and 100 mL) and an industry-made reagent HACH PathoScreen™ (HACH) (20 mL sample volume). These tests were studied, compared and evaluated based on three factors: accuracy, cost and practicality/ease of use. The factors and their associated criteria are presented in Table 1-4. It is important to note that Easygel®, Colilert and Petrifilm™ are tests that measure the presence of *E.coli*, which is a WHO indicator of fecal contamination. However, the H_2S test measures the presence of H_2S-producing bacteria, which has not been approved or verified to be a WHO indicator of fecal contamination (see Section 3.2.2).

The primary objective of this study was to verify the accuracy of the four field-based, microbiological tests: the H_2S tests (laboratory-made reagent for 10-, 20- and 100-mL sample volume, and industry-made HACH reagent), Easygel®, Colilert and Petrifilm™, by comparing them to Standard Methods of the enzyme substrate method using Quanti-Tray® and the membrane filtration method using m-ColiBlue® media through field testing in Capiz Province and at MIT. Concurrently, the H_2S-producing bacteria will be assessed as a potential new and valid indicator of fecal contamination. The second objective was to provide recommendations for a single P/A test (one of the H_2S tests, or Colilert) and a single enumerative test (Petrifilm™ or Easygel®) to be used in the field, based on three factors: accuracy, cost and practicality/ease of use. The third objective was to provide recommendations for a testing combination (similar to the EC-Kit) using one P/A test and one enumerative test based on three factors: accuracy, cost and practicality/ease of use.

Table 1-4. Factor and Criteria for Evaluating the Field-Based Tests.

Factor	Criteria
Accuracy	*Comparison with Standard Methods:* - H_2S/Easygel/EC-Kit (Colilert and 3M™ Petrifilm™) tests correlation with Quanti-Tray® test results - H_2S/Easygel/EC-Kit (Colilert and 3M™ Petrifilm™) tests correlation with membrane filtration test results *Statistical Analyses:* - Standard statistical analyses: True Results (TR), False Positives (FP), False Negatives (FN), Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) - 2x2 and 3x3 contingency tables - Chi-square and Fisher's exact tests - Scatter plot
Cost	*Fixed cost* - Equipment: vials/bottles *Variable cost* - Equipment - Reagents
Practicality/Ease of use	- Training for users: testers and readers - Ease of acquiring/making reagents - Ease of storage, transportation, and disposal of samples/tests - Incubation times - Use of electric incubator - Easy to read results

In this study, accuracy is defined as a combination of bias and precision of analytical procedure, which reflects the closeness of a measured value to a true value, obtained using Quanti-Tray® and membrane filtration (APHA, AWWA, WPCF, 2007). Validity, used to assess the suitability of H_2S-producing bacteria as an indicator of fecal contamination, will be defined as the ability of an indicator to accurately measure the concept it is intended to measure (i.e. fecal contamination) (Meier, Brudney, & Bohte, 2009).

In addition to accuracy, two other important factors will be considered: cost and practicality/ease of use. First, since the prime purpose of the EC-Kit tests, H_2S tests and Easygel® is to provide accurate microbial drinking water quality test results in a simple, low-cost manner to enable widespread testing of drinking water, particularly in developing countries, it follows that these tests should be inexpensive such that most, if not all, developing countries can afford to use them. Second, these tests will potentially be performed and read by people with little or no laboratory training, in remote areas with little or no electricity. Therefore these tests should be practical and easy to use: training for test users should be relatively quick and easy, acquiring/making reagents should be simple, samples/tests must be easily stored, transported and disposed of, the tests should require a short incubation times (ideally around 24 hours), the use of an electric incubator should not be mandatory, and the test results must be easy to interpret.

Only once the new tests (H_2S test, Easygel®, and EC-Kit: Colilert and Petrifilm™) have been assessed and compared can recommendations be made as to which are the better suited tests to use, either as single P/A or enumerative tests, or as a testing combination comprised of one P/A test and one enumerative test.

2. Drinking Water Indicators and the H_2S Bacteria

2.1. Drinking Water Indicators and Testing

2.1.1. Microbiological Contamination

In developing countries, microbiological contamination is the main source of drinking water pollution. The main microbiological risk associated with the ingestion of water is that it might be contaminated with human or animal faeces, which can be a source of pathogenic bacteria, viruses and protozoa (WHO, 2008). Many of these pathogens have severe health consequences from vomiting and diarrhoea, to typhoid, cholera, paraplegia, severe neurological illnesses and death. Microbial contamination is also particularly dangerous because it can spread very rapidly over a short period of time, so that by the time microbial contamination is detected, many people may already have been infected. As a result, water quality assessment methods have been developed in order to detect microbiological contamination quickly and accurately.

There are currently two main approaches to detecting microbial contamination. The first is direct detection, which means that pathogens (e.g. polio virus) are tested for directly. But this method is impractical because water samples would have to be tested for a wide variety of single pathogens that could be present in contaminated water. Furthermore, this method is time-consuming, expensive, and might carry some risks to the tester who is working directly with the pathogens themselves. For this reason, the standard practice for measuring fecal contamination in drinking water is testing for a non-pathogenic index organism or bacteria group considered indicative of fecal contamination (Sobsey & Pfaender, 2002). These bacteria can easily be isolated and quantified by a wide variety of simple bacteriological tests (Gerba, 2000). The following sections present different conventional microbiological indicators and their associated testing methods.

2.1.2. Conventional Indicator Organisms

An indicator organism (sometimes called indicator organism) is one that points to the presence of pathogenic organisms, such as an index of fecal pathogens (WHO, 2008), and indicates the presence of microbiological contamination in drinking water.

The current indicator organism of choice for fecal contamination is *E.coli*. Its popularity stems from the fact that it is the only member of the coliform group that is invariably found in coliforms in both human and warm-blooded animals. It also outnumbers other thermotolerant coliforms in human and animal feces (OECD, 2003). *E.coli* bacteria are a specific subset of thermotolerant coliform bacteria that possess the enzymes β-galactosidase and β-glucuronidase (WHO, 2008). The presence of *E.coli* indicates the presence of recent fecal contamination and detection should lead to further sampling and adequate treatment (WHO, 2008).

Analysis of indicator of fecal contamination, such as *E.coli*, provides a sensitive, although not the fastest, indication of contamination of drinking water supplies. Since the growth medium and the conditions of incubation, as well as the nature and age of the water sample, influence the types of species isolated and their concentration, microbiological examinations may have variable accuracies. This means that the standardization of methods and of laboratory procedures is of great importance if criteria for the microbial quality of water are to be uniform in different laboratories around the world (WHO, 2008).

Other popular indicator organisms of fecal contamination are thermotolerant coliform bacteria, which are a subset of total coliforms. Thermotolerant coliforms thrive in high concentrations of bile salts and ferment lactose at temperatures of 44-45°C. Thermotolerant coliforms include the genus *Escherichia* and some species of *Klebsiella*, *Enterobacter* and *Citrobacter* (OECD, 2003). Although thermotolerant coliforms are a less reliable index of fecal contamination than *E.coli* (the presence of thermotolerant bacteria can come from non-fecal sources), their concentrations are, in general, directly related to *E.coli* concentrations. Hence, their use for water quality testing is considered acceptable when no other method is available (OECD, 2003).

2.1.3. Alternate Index Organisms and Testing Methods

One of the greatest challenges in providing microbiologically-safe drinking water to communities around the world is the lack of adequate laboratory and testing facilities and trained personnel in the developing world to perform regular drinking water quality monitoring. In fact, although many testing methods currently exist (as indicated above), many of these require costly equipment, trained personnel, modern laboratory facilities, and are difficult to near impossible to apply in the field, in areas that lack access to electricity and clean drinking water. In an effort to overcome these limitations, low-cost, practical, and easy-to-use new types of indicators and tests that detect fecal contamination of drinking water have emerged. Some of these tests include P/A and MPN tests for *E.coli* or total coliforms.

A prominent, simple and low-cost index organism is the hydrogen sulfide (H_2S) producing bacteria, tested by the easy-to-use H_2S bacteria test, or more commonly called the H_2S paper strip test. Section 5.3. provides more detail on these new, microbiological, field-based tests.

2.2. What are H_2S-Producing Bacteria?

2.2.1. H_2S and the Sulfur Cycle

Sulfur is one of the ten most abundant elements on the planet and can be found in its various elemental, oxidized and reduced forms (Sobsey & Pfaender, 2002). The circulation of the various forms of sulfur is driven by the sulfur cycle, (Encyclopaedia Brittanica Inc., 2010) presented in Figure 2-1.

Sulfur occurs in all living matter as a component of certain amino acids and is abundant in the soil in proteins. The sulfur in the amino acids can be converted to sulfates ($SO4^{2-}$) by microorganisms or can be converted to H_2S by another group of soil microbes, sulfur-reducing bacteria. Sulfur-reducing bacteria, sometimes called H_2S-producing bacteria, are prominent in the sulfur cycle and obtain their energy by reducing elemental sulfur to a reduced form of sulfur: H_2S. Some, but not all of these bacteria are from the coliform bacteria group, while others are non-enteric, such as *Desulfovibrio* (Sobsey & Pfaender, 2002). If conditions are aerobic, H_2S is converted to sulfur and then to sulfate by sulfur bacteria (bacteria that transform H_2S into $SO4^{2-}$) (Encyclopaedia Brittanica Inc., 2010).

In surface and subsurface geohydrothermal environments (e.g. hot springs), H_2S is produced by sulfur respiration with hydrogen. H_2S is also produced by the mineralization or decomposition of amino acids and other organic forms of sulfur (Sobsey & Pfaender, 2002).

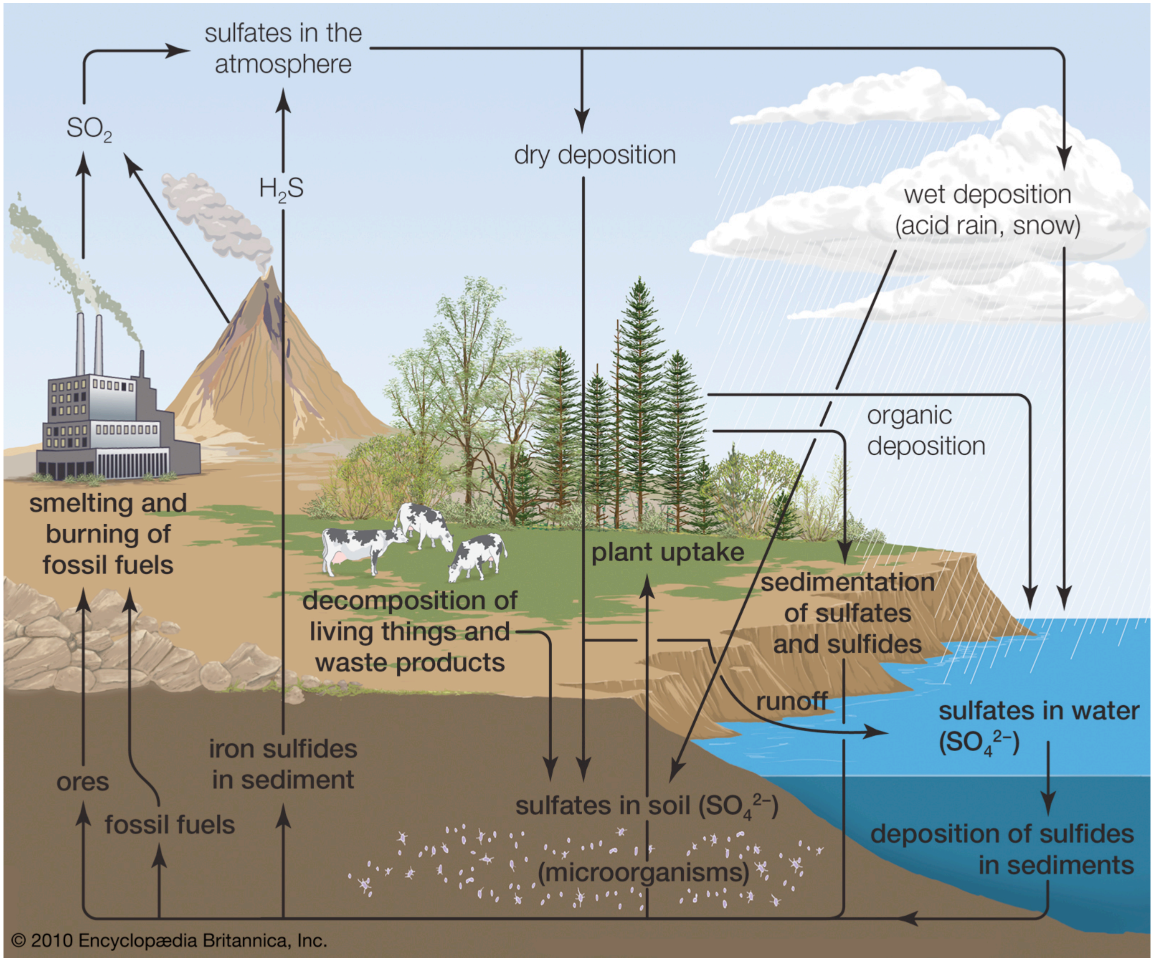

Figure 2-1. The sulfur cycle (Encyclopaedia Brittanica Inc., 2010).

2.2.2. Producers of H_2S

There are currently many known producers of H_2S, or H_2S-reducing bacteria. Some coliform bacteria (such as *Citrobacter*), some enteric bacteria (such as *Clostridium perfringens*), as well as other types of bacteria produce H_2S. However, only some types of *E.coli* produce H_2S.

Since H_2S is not a WHO-approved indicator, it is important to know how it relates to conventional indicators such as total coliforms (TC) and fecal coliforms. Figure 2-2 is a rough schematic of the relationship between the four groups of indicator organisms.

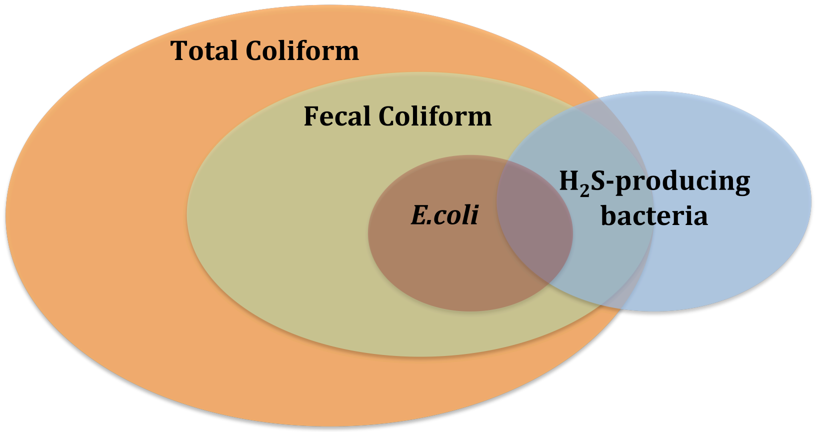

Figure 2-2. Illustration of the Relationship between Total Coliform, Fecal Coliform, *E.coli*, and H_2S-producing bacteria. (Adapted from Low (2002)).

Several investigations by Manja, Maurya, and Rao (1982), Ratto, Dutka, Vega, Lopez, and El-Shaarawi (1989), Kromoredjo and Fujioka (1991), Castillo, et al. (1994), and Grant and Ziel (1996) have attempted to identify the bacteria present in positive H_2S tests, in other words, bacteria that produce H_2S. They found that these bacteria were primarily various *Enterobacteriaceae* and *Clostridium perfringens*, such as *Citrobacter freundii*, *Enterobacter*, *Clostridia*, *Escherichia*, *Salmonella*, *Acinetobacter*, *Aeromonas*, *Morganella,* and some species of *Klebsiella* and *Edwardsiella*. The H_2S test was also shown to detect variants of H_2S-producing *E.coli*.

Although not all these bacteria are coliforms, they are organisms typically associated with the intestinal tract of warm-blooded animals (Sobsey & Pfaender, 2002). Indeed, several studies have shown good correlation between the presence of H_2S-producing bacteria and coliforms.

3. Literature Review: The H_2S test

3.1.1. History of the H_2S test

The H_2S test was first established by Manja, Maurya, and Rao (1982) during a hepatitis A outbreak in the city of Gawlior, India. They reported the development of a "simple, reliable field test for use by village public health workers" (Manja, Maurya, & Rao, 1982) to detect evidence of fecal contamination in drinking water. The H_2S bacteria test detects bacteria associated with fecal contamination due to the activity of these bacteria in producing hydrogen sulfide, which they found to be associated with the presence of coliforms in drinking water (Sobsey & Pfaender, 2002). Also, since the solubility of iron sulfide is particularly low, the test can dectet even small amounts of H_2S.

Many scholarly articles ((Kromoredjo and Fujioka (1991), Ratto, Dutka, Vega, Lopez, and El-Shaarawi (1989),Castillo, et al. (1994), Grant and Ziel (1996), Pillai, Mathew, Gibbs, and Ho, 1999), and Nair, Gibbs, and Mathew (2001) have since then tested and confirmed the original theory (Manja, Maurya, & Rao, 1982), and support the use of the H_2S bacteria test for drinking water.

3.1.2. How the H_2S bacteria test works

The H_2S test does not consistently measure the presence of either total coliform bacteria, or of fecal bacteria, or of specific groups of fecal bacteria such as *E.coli*. The test is based on measuring bacteria that produce H_2S (Sobsey & Pfaender, 2002), or more specifically, the test measures the presence of H_2S by its reaction with iron (as ferric ammonium citratite in the medium) to form a black iron sulfide (FeS) precipitate and a foul, "rotten egg" smell.

The presence of H_2S in a water sample is usually indicative of the presence of H_2S-producing bacteria, which has been shown to correlate with the presence of fecal coliforms (Manja, Maurya, & Rao, 1982). Although there are many variations of the H_2S bacteria test reagent, the procedure and main compounds remain the same as those stipulated by Manja, Maurya, & Rao (1982) for the original medium M1.

An added advantage of the H_2S test is that the test reagent includes sodium thiosulfate, which neutralizes chlorine present in a water sample. This means that the H_2S test is a suitable microbiological test for chlorinated water supplies, unlike other microbiological tests that cannot be used for such supplies.

In 1994, Venkobachar et al. suggested that the addition of L-cystine (medium M2), a sulphur-containing amino acid, might increase the sensitivity of the H_2S strip. His research, in addition to findings by Pillai, Mathew, Gibbs, & Ho (1999), showed that the addition of L-cystine was highly beneficial: it improves the contamination detection rate, especially at lower concentrations, and yields more reliable results, especially at lower temperatures.

Furthermore, the originial medium established by Manja, Maurya, and Rao (1982) used 1 mL of Teepol. However, since Teepol is hard to obtain, Grant and Ziel (1996) replaced it by lauryl sulfate salts (or sodium lauryl sulfate).

Appendix A gives a step-by-step procedure on how to make the H_2S reagents used in this study, how to conduct the tests and interpret results.

3.1.3. Historical development and study of H_2S bacteria test

Manja, Maurya, & Rao (1982) initially developed the H_2S test to detect fecal contamination of drinking water in several cities in India. The test was developed to detect the production of H_2S by enteric bacteria by the formation of a black precipitate from the reaction of H_2S with iron in the medium (Sobsey & Pfaender, 2002). Water samples containing 10 or more coliform bacteria per 100 mL, as assessed by the MPN test, and those turning black in the H_2S test (20-mL sample volume) were graded as unsatisfactory for consumption. The positive samples were cultured in order to isolate and identify the organisms that produce H_2S.

Since then, many investigators have tested the H_2S bacteria test to gauge its accuracy, and others have recommended modifications to improve its performance. Some of the tests and modifications reported in the literature are summarized below.

Ratto et al. (1989) evaluated the H_2S test (20-mL sample) at incubation temperatures of 22°C and 35°C, and compared it to total and fecal coliform P/A and MPN tests. In total, 20 water samples from five different distribution line sources in Lima, Peru were tested. The research concluded that the H_2S test and fecal coliform P/A test were equally or more sensitive than the total coliform MPN tests at pollution indicator bacteria. Ratto et al. (1989) also concluded that the H_2S bacteria test would be an ideal procedure for isolated water supplies and where laboratory facilities do not exist.

Kromoredjo & Fujioka (1991) evaluated and compared the H_2S paper strip test (20-mL sample volume) to the lauryl tryptose + 4 methyl-umbelliferyl-β-d-glucoronide (LTB+MUG test), and the Colilert test (MPN method). The objectives of this study were to determine the microbial quality of water in the distribution system of Banjarmasin, Indonesia; and to assess the feasibility and reliability of using these aforementioned three microbial tests as accurate methods to monitor drinking water supplies in developing countries. This study concluded that all three methods closely correlated and appeared to be equally effective in their ability to detect fecal contamination. In assessing the most appropriate method to be used in developing countries, the authors supported the use of the H_2S bacteria test because of its accuracy (results correlated with other standard methods), low-cost, zero electricity use (does not require a refrigerator or incubator), ease of use, and shorter incubation periods.

Castillo et al. (1994) evaluated the feasibility of the H_2S paper strip method (100-mL sample volume) by testing drinking water samples from disinfected and non-disinfected sources in three regions of Chile, and comparing those test results to results obtained using the total coliform MPN and coliphage tests. The H_2S test produced 10% more positive samples than the total colifom MPN test, which included samples that were positive for *Clostridium*. Other bacteria detected by the H_2S paper strip test included *Klebsiella*, *E.coli*, *Clostridium*, and *Salmonella*. It was concluded that since the H_2S bacteria test yielded slightly more positive results (some containing *Clostridium*), it could possibly offer slightly better protection to consumer. Also, the H_2S bacteria test results gave similar results at both 32°C and 35°C, indicating that an incubation temperature within that range is not critical to the functioning of this test. Lastly, it was concluded that the sensitivity, simplicity and low cost of the H_2S bacteria test was applicable to tropical and subtropical waters.

Venkobachar et al. (1994) assessed bacteriological water quality using a modified H_2S paper strip test (original media + L-cystine) for a 20-mL sample volume. Several water samples from Indian rural villages were collected and tested using the original H_2S paper strip test, the modified H_2S paper strip test, and total coliform and fecal coliform MPN tests. Correlation Analyses (TR, FP, FN) indicated that the addition of L-cystine to the original H_2S medium reduced the time required for assessing bacterial contamination and also increased sensitivity.

Grant & Ziel (1996) changed the H_2S paper strip test by replacing 1 mL of Teepol, as stipulated by (Manja, Maurya, & Rao, 1982) with lauryl sulfate salts (or sodium lauryl sulfate), and by using a 100-mL sample volume. They also evaluated the effectiveness of the H_2S bacteria test using water samples from a temperate region, since earlier research had been conducted with samples from tropical and sub-tropical regions. The H_2S bacteria test results were compared with coliform P/A test, coliform membrane filtration media, and *Clostridium perfringens* medium. In this study, the H_2S medium was not absorbed onto paper, instead a six-fold concentrated medium was used. Also, the original medium used Teepol 610, which is no longer manufactured. So this reagent was replaced by a similar surfactant: lauryl sulphate sodium salts. Results of this research concurred with Manja, Mauray, & Rao (1982) that the H_2S bacteria test is an effective alternative procedure for monitoring drinking water quality.

Pillai et al. (1999) determined the reliability of the H_2S test (20-mL sample volume) for detecting fecal contamination in drinking water. The research used diluted samples of feces and looked at the influence of temperature (0°C to 47°C), contamination level (1 to 1000 CFU/100mL) and modifications to the H_2S media (M1, M2 and the addition of yeast extract to the M1 media) on incubation period. The study did not look at field samples of water. The results showed that H_2S bacteria test was most effective when carried out at temperatures between 22°C and 44°C; and that an increase in incubation period usually correlated with a decreasing concentration of fecal pathogens. There was also a significant difference in incubation period between the three different H_2S media used. The addition of L-cystine (M2 media) was found to be the most effective, decreasing incubation periods by up to 50%.

Rijal et al. (2000) compared two versions of the H_2S bacteria test: a paper strip MPN test (20-mL sample volume) and a newly-developed membrane filter enumeration on agar medium. These results were compared to total coliform and *E.coli* testing results in samples of rainwater, groundwater and stream water. The results of fecal contamination detection showed that both versions of the H_2S bacteria test yielded similar results to the *E.coli* tests. However, total coliforms were detected in more samples than H_2S bacteria and *E.coli* tests. The tests in this study were also used to determine the efficacy of a solar disinfection system. The indicator-reduction results obtained were similar for all fecal indicators used. Rijal et al. (2000) concluded that the H_2S bacteria test was an appropriate and reliable measure to determine the quality of drinking water and the efficacy of treatment methods.

Nair et al. (2001) assessed the suitability of the H_2S test (20-mL sample volume) for testing untreated and chlorinated water supplies. Water samples from rainwater, borewater and catchment sources were tested using the H_2S bacteria test (M1 and M2 media), which was compared to the membrane filtration test. The test also compared the tests' sensitivity (ability of a test to detect a true positive result) and specificity (the ability of a test to detect a true negative result) for different water sources. The research concluded that, in developing countries, the H_2S bacteria test would be a good test to identify microbial contamination, whereas in other regions, the H_2S method could be used as a screening test in household rainwater tanks or remote communities where no other facilities are available.

HACH Company also produces a PathoScreen™ powder medium that detects the presence H_2S-producing bacteria. The medium is dehydrated, sterilized and packaged in powder pillows and is available in both the P/A and MPN testing.

3.2. H_2S-producing-bacteria: a new indicator

3.2.1. WHO guidelines

The current practice of testing for indicator and index organisms as signals of fecal contamination is a well-established practice in water quality monitoring. The WHO (2008) has defined the current criteria of an ideal or preferred indicator of fecal pollution. According to them, the essential criteria of a fecal indicator/index are the following (WHO, 2008):

- Be universally present in feces of human and animals in large numbers;
- Not multiply in natural waters;
- Persist in water in a similar manner to fecal pathogens;
- Be present in higher numbers than fecal pathogens;

- Respond to treatment processes in a similar fashion to fecal pathogens; and
- Be readily detected by simple, inexpensive methods.

Therefore any indicator of fecal contamination should be judged and compared according to the above-mentioned WHO (2008) criteria. It is important to note that no one indicator meets all these criteria. Thus it is usually important to consider a variety of indicator microorganisms to assess fecal contamination in a given water sample.

3.2.2. H_2S-producing bacteria as a WHO indicator

There has been much debate as to whether or not H_2S-producing bacteria are suitable indicators, and meet the criteria for an ideal or preferred indicator of fecal contamination. In fact, many reports have stated that the H_2S bacteria test is a suitable indicator of microbiological contamination of drinking water, even though there appears to be no analyses and "expert judgment" that went into the development of H_2S-producing bacteria as a reliable and accurate indicator, or in the development of the H_2S test as a P/A test (Sobsey & Pfaender, 2002).

The following lists the essential criteria of an ideal or preferred index of fecal contamination as per the WHO (2008) and the studies and findings that have addressed them.

- Be universally present in feces of human and animals in large numbers

Pillai et al. (1999) studied the reliability of the H_2S bacteria test by using the test on a variety of fecal dilutions. The results showed that H_2S-producing bacteria were present in large numbers in human feces. Indeed, the H_2S paper strip turned black at dilutions of 1 in 1,000,000.

Also, positive and negative H_2S test results correlated with positive and negative test results, respectively, from other microbiological tests. This showed that the presence and absence of H_2S-producing bacteria correlates to the presence and absence, respectively of other indicators of fecal contamination (Castillo, et al., 1994)(Grant & Ziel, 1996)(Kromoredjo & Fujioka, 1991)(Manja, Maurya, & Rao, 1982)(Nair, Gibbs, & Mathew, 2001)(Pillai, Mathew, Gibbs, & Ho, 1999) and(Ratto, Dutka, Vega, Lopez, & El-Shaarawi, 1989).

- Not multiply in natural waters

Enteric bacteria such as *Citrobacter*, *Salmonella*, *Proteus* and some species of *Klebsiella* produce H_2S (Manja, Maurya, & Rao, 1982). These members of the coliform group have been observed to regrow in natural surface and drinking water distribution systems (Gleeson and Grey (1997) in Gerba (2000)). The die-off rate of coliform bacteria primarily depends on the initial concentration of coliforms in the water and temperature. So a large amount of coliforms in high-temperature (37°C) waters would trigger an increase in number of fecal coliforms in natural waters. Indeed this has been shown to occur in eutrophic tropical waters (Gerba, 2000). This is also true of other indicators such as *E.coli* and total coliforms.

- Persist in water in a similar manner to fecal pathogens

The coliforms identified by Manja, Maurya, & Rao (1982) as H_2S-producing are also fecal pathogens that can cause detrimental health effects from gastroenteritis, septicaemia, bacteriaemia and typhoid. Therefore they would also persist in water in a similar manner to fecal pathogens.

- Be present in higher numbers than fecal pathogens

This criterion has, as of now, remained untested.

- Respond to treatment processes in a similar fashion to fecal pathogens

Rijal et al. (2000) tested and compared results of H_2S bacteria test (MPN and membrane filtration), *E.coli* and total coliform tests for a solar disinfection system. The study showed that H_2S-producing bacteria behaved in a similar manner to *E.coli* and total coliform indicators and therefore can be expected to behave in a similar fashion to fecal pathogens. Also, it is worth mentioning that the H_2S test detects *Clostridium perfringens*, which is one of the more resistant indicators of fecal contamination and can be found in drinking waters when no coliform is found (Sobsey & Pfaender, 2002).

- Be readily detected by simple, inexpensive methods

The H_2S-producing bacteria are detected by the H_2S bacteria test. The chief advantage of this test is that the reagents are inexpensive and widely accessible, the test does not require electricity (for a refrigerator or incubator), test results are seen rapidly, often after 12 to 15 hours of incubation (Kromoredjo & Fujioka, 1991).

Furthermore, all reports indicate that it is by far the most inexpensive method for testing for fecal contamination (Sobsey & Pfaender, 2002).

3.2.3. Additional H_2S bacteria test verification

Although the H_2S bacteria test meets most of the WHO (2008) criteria, the test still requires some additional verification. For example, no research has yet studied the relationship between concentrations of H_2S-producing bacteria and concentration of fecal pathogens. Also, it would be useful to understand the manner in which H_2S-producing bacteria respond to disinfection methods, such as disinfection by chlorine, ozone, solar or boiling, and compare that response to other fecal pathogens. Finally, one of the main criticisms of the H_2S bacteria test is that it might potentially detect H_2S-producing bacteria not associated with fecal contamination, since, for example, H_2S may be naturally present in groundwater. Therefore, a study, which identifies what bacteria and pathogens the test actually detects, would be essential in order to establish the accuracy and validity of the H_2S bacteria test.

4. Drinking Water Sources and Types

4.1. Global Drinking Water Sources

4.1.1. Millennium Development Goals

In September 2000, the United Nations (U.N.) established a set of eight "Millenium Development Goals" (MDGs) that set quantitative benchmarks to reduce extreme poverty in all its form by 2015 (U.N., 2009). It included goals to eradicate extreme poverty and hunger, to achieve universal primary education, to promote gender equality and women's empowerment, to reduce child mortality, to combat diseases, to ensure environmental sustainability and to promote a global partnership for development.

4.1.2. U.N. Designation of Drinking Water Sources

The drinking water target (Target 7.C) is to "halve, by 2015 the proportion of people without sustainable access to safe drinking water and basic sanitation." (UNDP, 2010), where "access to safe water" refers to the percentage of the population with reasonable access to an adequate supply of safe water in their dwelling, or within a convenient distance of their dwelling (UNDP, 2003). In order to help track progress of the MDGs' targets, international and national statistical experts selected relevant indicators to be used to assess progress. The indicator for Target 7.C is the "proportion of population using an improved drinking water source" (UNDP, 2003). Improved and unimproved drinking water source types are presented in the Drinking Water Ladder in Table 4-1. 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. It is also important to note that the MDG target for drinking water is divided into urban and rural populations, in order to highlight urban and rural disparities, which would otherwise be masked by aggregate figures (WHO/UNICEF, 2008).

Today, approximately 87% of the world's population uses an improved drinking water source: 54% use a piped connection, and 33% use other improved drinking water sources. This represents an increase of 1.6 billion people with improved access since 1990 (WHO/UNICEF, 2010).

Table 4-1. Drinking Water Ladder (Adapted from WHO/UNICEF (2008)).

Drinking water supply	Drinking water supply type
Unimproved	**Unimproved drinking water sources:** Unprotected dug well Unprotected spring Cart with small tank/drum Surface waters (river, dam, lake, pond, stream , canal, irrigation channels) Bottled water
Other improved	**Other improved drinking water sources:** Public taps or standpipes Tube wells or boreholes Protected dug wells Protected springs Rainwater collection
Improved	**Piped water on premises:** Pipes household water connection located inside the user's dwelling, plot or yard.

4.2. Capiz Province Drinking Water Sources

4.2.1. Drinking Water Uses in the Philippines

In 1999, the total renewable water resources in the Philippines were estimated to be 479 km^3 (CIA, 2009), or roughly 4,900 m^3 per capita. As such the Philippines does not suffer from freshwater stress (<10% freshwater withdrawal as percentage of total available) (UNEP, 2002), although it is often prone to floods and drought.

In 2000, freshwater withdrawals were estimated to be approximately 29 km^3 per year; with a breakdown of 17%, 9% and 74% for domestic, industrial and agricultural uses, respectively. Agriculture exerts significant pressure on the freshwater resources. As a matter of fact, in 2003, an estimated land area of 15,500 km2 (5% of the total land area of the country) was being irrigated. Furthermore, the use of irrigation is increasing, as threats of climate change and El Niño loom, causing droughts and below average rainfall. In light of these facts, the President, Gloria Macapagal-Arroyo, has recently called for early completion of a major national irrigation project. Thus, while the country overall remains one of water abundance, the uneven spatial and temporal distribution are key factors impacting emerging water use trends in the country (Gov.Ph, 2009). It is also important to note that water is also unevenly distributed among the rich and poor, where wealth is directly related to access to water (Table 4-2).

Table 4-2. Rich-Poor Disparities in the Philippines.
(UNICEF, 2008)

Wealth quintile	Water access (%)
Poorest	67
2nd	91
3rd	96
4th	96
Wealthiest	98

4.2.2. Drinking Water Sources in the Philippines

According to UNICEF/WHO (2008), in 2006, 93% of the Philippines' total population used an improved drinking water source: 53% used a piped connection, and 40% used other improved sources. This represented a 10% increase from 1990.

In urban areas, however, 69% use a piped connection, and 27% use other improved drinking water sources. This represents a 4% increase from 1990. In rural areas, 88% of the population use improved water sources: 24% use a piped connection and 64% use other improved drinking water sources. This represents a 13% increase since 1990 (WHO/UNICEF, 2008). The drinking water coverage data (the proportion of a population using a particular drinking water source) for the Philippines is presented in Figure 4-1 and Figure 4-2. These figures highlight the urban/rural drinking water source disparity.

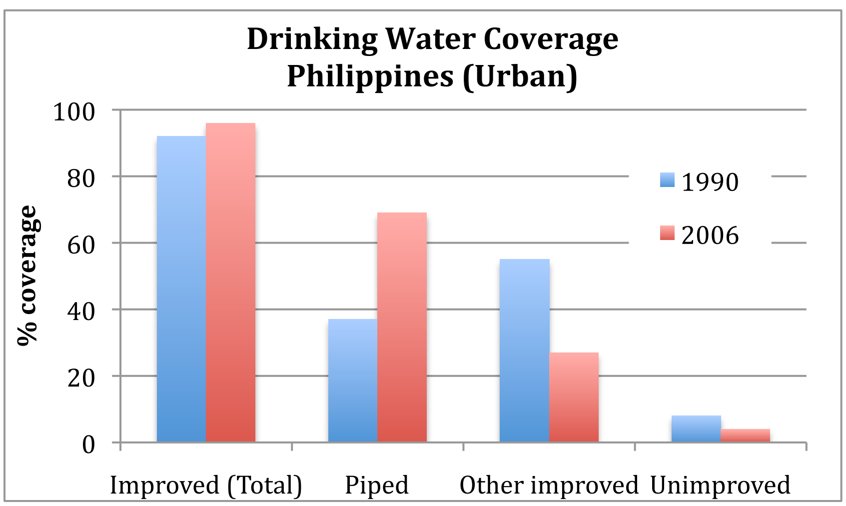

Figure 4-1. Drinking Water Coverage - Philippines (Urban Population). (WHO/UNICEF, 2008)

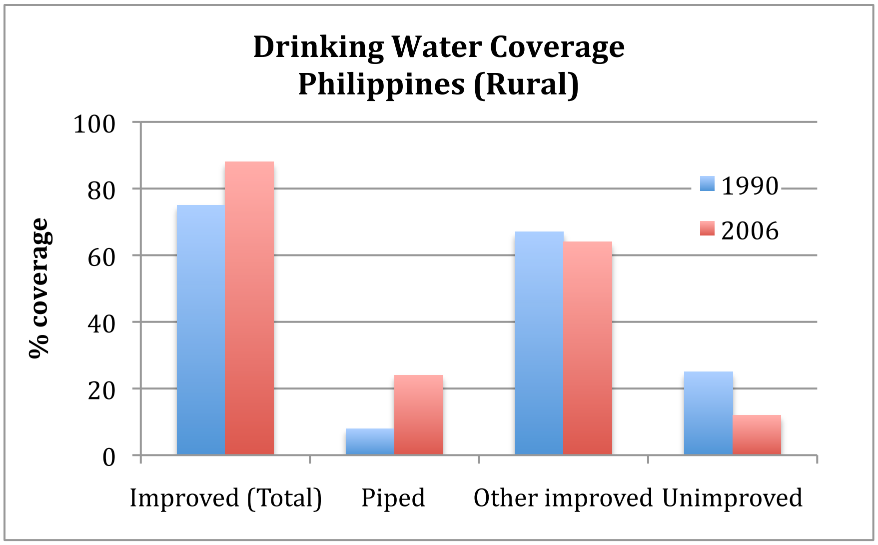

Figure 4-2. Drinking Water Coverage - Philippines (Rural Population). (WHO/UNICEF, 2008)

4.2.3. Drinking Water Sources in Capiz Province

According to the National Statistical Coordination Board (NSCB) (2009) of the Philippines, as of 2000, 119,000 households in Capiz (or 92% of the Capix population) have access to an improved drinking water supply.

The Capiz PHO currently uses four water source categories to designate their drinking water source types: Levels 1, 2 and 3, and Doubtful sources. Levels 1 through 3 fall under the U.N.'s "improved" category, whereas Doubtful sources are "unimproved." A summary of the Capiz Province and corresponding U.N. designation are presented in Table 4-3. These designations and abbreviations, as defined by the Capiz PHO, will be used throughout this study to describe water source levels and types.

Table 4-3. Capiz PHO Water Source Designation and Corresponding U.N. Designation Category.

U.N. Designation Category	Capiz PHO Designation	
	Category	**Source Type**
Improved	Level 3 (piped connection on premises)	▪ Water district ▪ Local water utilities administration ▪ *Barangay* waterworks system
	Level 2	▪ Gravity protected spring with pipe distribution to communal tap stands ▪ Deep well with pump, with pipe distribution to communal tap stands
	Level 1	▪ Shallow well pump ▪ Jetmatic pump with or without motor ▪ Deep well pump ▪ Protected dug well ▪ Protected spring without distribution ▪ Rainwater catchment (ferro-cement tank)
Unimproved	Doubtful	▪ Open dug well ▪ Unprotected spring ▪ Surface water (rivers, streams, creeks) ▪ Others

4.3. Sampling Water Sources in Capiz

The water sources that were tested using the EC-Kit and Quanti-Tray® tests (by the PHO), and using the H_2S tests and Easygel® (by the author), were selected using a stratified sampling methodology. In other words, samples were not randomly selected from the entire water level spectrum (Doubtful to Level 3), but were rather selected within their own subpopulation (water level). This means that a set number of samples per subpopulation was first determined, and then samples within their subpopulation were randomly selected for testing. This procedure intentionally skewed the sample selection process towards Doubtful, Level 1 and known contaminated sources. In fact, most of the sources sampled in January 2010 and tested by the H_2S tests and/or Easygel®, and EC-Kit and Quanti-Tray®, were Doubtful (20%) and Level 1 (50%) sources. The chief reason for this research design was because each test represented a significant investment on the part of the PHO, and their intention was to target sources that were more likely to yield contaminated results, in order to be able to set priorities for present and future actions.

5. Study Procedure and Microbiological Test Methods

5.1. Research Plan and Methodology

The research plan and methodology for this study were comprised of the following steps: (1) a review of the main literature of the H_2S test; (2) preliminary laboratory testing of different water samples collected in the Boston/Cambridge area using the H_2S test and different sample volumes; (3) development of an overall research plan and sampling protocol for the Philippines; (4) field testing in the Philippines for the H_2S and Easygel® tests, concurrent with the PHO sampling and testing using Quanti-Tray® and EC-Kit tests; (5) additional testing at MIT, including H_2S test, Easygel®, EC-Kit, Quanti-Tray® and membrane filtration of Charles River collected at the MIT Sailing Pavilion (Building 51).

5.1.1. Literature Review

As a starting point, a comprehensive review of the literature on the H_2S test, from Manja, Maurya, and Rao (1982) to Sobsey and Pfaender (2002), was performed. The purpose of the literature review was to provide background information on the purpose of the H_2S test, its history, use, accuracy, limitations and criticism. Furthermore, the literature review was undertaken in order to note improvements that had been made to the original H_2S media and which could be introduced for field testing in Capiz Province.

5.1.2. Preliminary Laboratory Testing at MIT, November 2009

In November 2009, preliminary tests were conducted on the Charles River, Boston, MA, Redd's Pond, Marblehead, MA and rainwater samples from Marblehead, MA. The H_2S media used for these tests included the original medium (M1) developed by Manja, Maurya, and Rao (1982), the M2 medium (M1 + L-cystine) developed by Venkobachar, Kumar, Talreja, Kumar, and Iyengar (1994) and the industry-made HACH test reagent. The purpose of the preliminary laboratory testing was to first, familiarize the author with making the H_2S test strip reagent, conducting the EC-Kit and Quanti-Tray® tests, and reading the test results. The water samples tested during these studies were the Charles River in Boston, MA and Redd's Pond in Marblehead, MA (10 samples from each source were tested, in addition to 2 blanks and 1 duplicate). It is important to add that these tests were carried out using expired H_2S test strip ingredients, which nonetheless provided data adequate for the learning process, but the test results were not included in the final data set.

5.1.3. Development of an Overall Research Plan

During Fall 2009, an overall research plan was developed. It was established that during January 2010, in Capiz Province, 165 samples would be tested using the H_2S tests (laboratory-made and HACH), and 50 samples would be tested using the Easygel® test. It must be noted that the water samples tested by the H_2S tests and Easygel® represent a subset of all water samples tested under the water quality testing program in the PHO. Therefore the samples tested by the H_2S tests and Easygel® were also tested by the EC-Kit and Quanti-Tray® by the PHO. The samples tested by the H_2S tests and Easygel® were chosen such that most municipalities and water sources of Capiz Province would be represented, and such that the majority of the samples collected would come from high-risk sources (Doubtful and Level 1 sources).

Upon return from the Philippines, it was determined that additional laboratory testing of the field-based microbiological tests would be important in correctly determining the accuracy of the different field-based, microbiological tests (see Section 1.6.6.).

5.1.4. Laboratory-Made H_2S Test Reagent Preparation

In December 2009, the laboratory-made H_2S test reagents were prepared at the MIT Environmental Engineering Laboratory. Since the M2 medium yielded better and more accurate results than the M1 medium, it was decided that the H_2S test used throughout this study, both in the Philippines and at MIT, would be the M2 medium. A series of 165 test reagents was prepared for each of the 10-, 20- and 100-mL sample volume tests. For the 10- and 20-mL H_2S test reagents, the paper strip reagent was prepared in the 10- and 20-mL vials, respectively. Throughout this study, the 10- and 20-mL laboratory-made H_2S tests were conducted in glass vials. However, the 100-mL H_2S test reagents were prepared without vials. These paper strip reagents were stored in sterile, sealable, plastic bags until the point of use. Throughout this study, the 100-mL laboratory-made H_2S tests were conducted in sterile sampling bags.

5.1.5. Field Testing in Capiz Province, January 2010

The MIT team's field testing in Capiz Province is presented in detail in Section 5.2.1.

5.1.6. Additional Laboratory Testing at MIT, April 2010

Since the test results from the field-based tests conducted in Capiz were compared to one standard method only (Quanti-Tray®), it was decided that additional laboratory testing using a second standard method (membrane filtration) would be beneficial in confirming the accuracy results obtained by comparing the field-based microbiological tests to Quanti-Tray®. Furthermore, since only a few samples (43) were tested by the Easygel® test in Capiz, it was determined that a larger Easygel® test sample size would help yield more statistically significant results. Therefore, during April 2010, water samples from the Charles River, Cambridge, MA, were collected and analyzed using the laboratory-made H_2S tests (10-, 20- and 100-mL sample volumes), the HACH test (20-mL sample volume), Easygel® and EC-Kit. These tests were compared to Quanti-Tray® and membrane filtration.

5.2. Description and Scope of Experiments

Data collection for the H_2S tests and Easygel® verification began in January 2010, in Capiz Province, Philippines. Water samples from 16 municipalities and Roxas City were tested for microbiological contamination using Quanti-Tray®, EC-Kit, H_2S tests and/or Easygel®. Additional laboratory testing also took place in April 2010, at MIT, Cambridge, where Charles River water samples were collected, tested and analyzed. The microbiological tests used in this study are presented in detail in Section 5.3.

5.2.1. Fieldwork in Capiz Province

In January 2010, the MIT team arrived in Roxas City and began the drinking water quality assessment program for Capiz Province in collaboration with the MIT team. In addition to the EC-Kit and Quanti-Tray® testing undertaken by the PHO, a selection of samples collected by the MIT team in January 2010 was also tested using the H_2S tests (laboratory-made reagent for 10-, 20- and 100-mL sample, and HACH for 20-mL sample). During the third week of January 2010, Susan Murcott and the author decided to test Capiz Province water samples using another field-based test: Easygel®. So a selection of the samples collected during the last 2 weeks of January 2010 was also tested using Easygel®. Table 5-1 presents the sampling schedule followed in Capiz Province, Philippines, and the number of samples collected per day, per *barangay*, per municipality and per analytical test.

The equipment, supplies and training for the PHO to perform these tests were obtained during January through November 2009. Hence the overall water quality testing program undertaken by the Capiz PHO began in December 2009. The objective was to collect, test and analyze 1,000 sets of water samples, comparing several different test methods, by March 2010, at which point the water quality testing program would be completed. All 16 municipalities plus Roxas City were included in the overall population; but at the *barangay* level, the water sources to be sampled were randomly selected, and were usually households, or communal sources or tap stands. The Capiz PHO established a stratified sampling sample selection method (see section 4.3.), where sample selection was skewed by first singling out some areas of Roxas City, Panay, Panitan for chlorine testing, and only after determining there was no chlorine residual, performing microbiological testing; and second, by biasing the sample selection towards Doubtful, Level 1 and known contaminated sources.

The municipal SI's collected water samples continuously from December 10, 2009 to March 24, 2010, and carefully labeled the samples based on a pre-determined labeling system established by the Capiz Province PHO. Appendix F provides more information on the labeling procedure. In January 2010, the MIT team accompanied the SI's for most of that month's sampling, and helped with some of the Quanti-Tray® and EC-Kit sampling. Sample preparation, processing, and incubation were done at the water quality laboratory at the Roxas Memorial Hospital in Roxas City. The Quanti-Tray® tests were performed by medical technicians at Roxas Memorial Hospital, Jane Delos Reyes, Sanitary Engineer at the Capiz PHO, and, in January 2010, with the help of MIT teammate Patty Chuang. Sanitary engineers from municipal health offices processed and interpreted the EC-Kit tests. The author performed the H_2S tests; while Susan Murcott and Patty Chuang performed the Easygel® tests.

The Capiz Province results presented in this thesis are of the samples collected in January 2010 that were tested using the H_2S tests and/or Easygel®, with their concurrent Quanti-Tray® and EC-Kit tests. Chuang (2010) summarized and analyzed the Quanti-Tray® and EC-Kit test results obtained for the entire sampling program (from December 2009 to March 2010) representing the work of the overall PHO/MIT team related to the water quality test program.

5.2.1.1. H_2S Tests

In total, 164 drinking water samples were collected and tested by the author using the H_2S test: 33 Doubtful, 91 Level 1, 15 Level 2, and 25 Level 3 sources. Drinking water sources were sampled from the following 12 municipalities and Roxas City: Cuartero, Dao, Dumalag, Dumarao, Ivisan, Maayon, Mambusao, Pilar, Pontevedra, President Roxas, Sapian, and Tapaz.

5.2.1.2. Easygel®

In total, 43 drinking water samples were collected and tested using the Easygel® test: 13 Doubtful, 13 Level 1, 12 Level 2, and 5 Level 3 sources. Drinking water sources were sampled from the following 7 municipalities: Dao, Dumarao, Jamindan Mambusao, Panay, Sigma, and Tapaz.

5.2.1.3. EC-Kit

In total, 176 drinking water samples were collected and tested using the EC-Kit: 43 Doubtful, 89 Level 1, 20 Level 2, and 25 Level 3 sources. Drinking water sources were sampled from the following 15 municipalities and Roxas City: Cuartero, Dao, Dumalag, Dumarao, Ivisan, Jamindan, Maayon, Mambusao, Panay, Pilar, Pontevedra, President Roxas, Sapian,

5.2.1.4. Quanti-Tray®

In total, 178 drinking water samples were collected and tested using Quanti-Tray®: 43 Doubtful, 90 Level 1, 20 Level 2, and 25 Level 3 sources. Furthermore, drinking water sources were sampled from the following 15 municipalities and Roxas City: Cuartero, Dao, Dumalag, Dumarao, Ivisan, Jamindan, Maayon, Mambusao, Panay, Pilar, Pontevedra, President Roxas, Sapian, Sigma, and Tapaz.

Table 5-1. Sampling Schedule in Capiz Province, January 2010.

Schedule	Municipality	*Barangay* Name	*Barangay* Number	Source Level[1]	Number of Samples Collected per Analytical Test			
					Quanti-Tray®	EC-Kit	H_2S test	Easygel®
06-Jan-10	Sapian	Bilao	B1	L1	5	5	5	NT
		Lonoy	B2	L1	5	5	5	NT
07-Jan-10	Pontevedra	Ameligan	B3	L1	2	2	2	NT
		Nelia Manaay	B23	D (1) L1 (1)	2	2	2	NT
		Guba	B10	D (3) L1 (1)	4	4	4	NT
		Tacas	B25	L1	1	1	1	NT
		Rizal	B20	L1	1	1	1	NT
	President Roxas	Hanglid	B3	D (1) L1 (2)	3	3	3	NT
		Poblacion	B1	D (4) L1 (1)	5	5	5	NT
08-Jan-10	Maayon	Poblacion Ilawod	B5	D	3	3	3	NT
		Poblacion Tabuc	B6	L1	2	2	2	NT
		Palaguian	B4	L1	1	1	1	NT
		Quinat-Uyan	B1	L1	1	1	1	NT
		Batabat	B2	L1	1	1	1	NT
	President Roxas	Cubay	B13	L1	2	NT	2	NT
	Pilar	San Pedro	B4	D	5	5	5	NT
12-Jan-10	Dumalag	San Roque	SR	L1	7	7	7	NT
		Santa Cruz	SC	L1	8	8	8	NT
13-Jan-10	Dumalag	Concepcion	BC	L1	1	1	1	NT
		Dolores	BD	L3	5	5	5	NT
		Poblacion	BP	L3	5	5	5	NT

		San Angel	BSA	D	4	4	4	NT
14-Jan-10	Ivisan	Santa Cruz	B2	L1	5	5	5	NT
		Poblacion Ilaya	B3	L1	5	5	5	NT
	Roxas City	Lanot	B1	L1	4	4	4	NT
		San Jose	B2	D (1) L1 (1)	2	2	2	NT
		Jumaquicjic	B3	L1	4	4	4	NT
15-Jan-10	Ivisan	Matnog	B4	L2	5	5	5	NT
		Agmalobo	B5	D	5	5	5	NT
18-Jan-10	Cuartero	Bitoon Ilaya	B4	D	1	1	1	NT
		Bitoon Ilawod	B3	L1	5	5	5	NT
		Poblacion Ilawod	B9	L1	2	2	2	NT
		Poblaction Tacas	B9	L1	4	4	4	NT
19-Jan-10	Dumarao	Codingle	B1	L3	5	5	5	5
		Poblacion Ilaya	B2	L3	5	5	5	NT
		Poblacion Ilawod	B3	L3	5	5	5	NT
20-Jan-10	Tapaz	San Julian	B6	L2	5	5	5	4
		San Nicolas	B7	L2	5	5	5	4
21-Jan-10	Dao	Matagnop	B5	L1	9	9	10	6
		Poblacion Ilawod	B4	L1	2	2	2	2
		Nasunogan	B6	L1	1	1	1	NT
		Manhoy	B7	L1	1	1	1	1
22-Jan-10	Mambusao	Caidquid	B1	D (5 and 4) L1 (5)	10	10	10	9
25-Jan-10	Panay	Magubilan	B4	D	5	5	NT	5
26-Jan-10	Sigma	Parian	B1	L2	5	5	NT	4
27-Jan-10	Jamindan	Agambulong	B1	D	5	5	NT	3
				TOTAL	**178**	**176**	**164**	**43**

NT: Not tested

[1] Source Levels are presented as per the Capiz PHO designation: Doubtful (D), Level 1 (L1), Level 2 (L2) and Level 3 (L3). Numbers in parentheses indicate the number of samples collected per water source level for a given *barangay*.

5.2.2. Laboratory Studies at MIT

On April 4, 2010, laboratory studies were conducted at MIT using the H_2S tests (10-mL, 20-mL, 100-mL and HACH medium), Easygel®, EC-Kit, Quanti-Tray® and membrane filtration. The water samples tested were dilutions of the Charles River, plus four undiluted samples of Charles River, and two samples of de-ionized water (blanks). A total of 9 dilutions were prepared, with 4 water samples tested per dilution for every test conducted. A total of two blanks and two duplicates were also prepared. Table 5-2 presents the dilutions and number of samples that were tested per analytical test.

Table 5-2. Samples Prepared and Tested During MIT Laboratory Studies on April 4, 2010.

Dilution[1]	Source[2]	Source Level[3]	Number of Samples Collected per Analytical Test				
			Quanti-Tray®	EC-Kit	H_2S test	Easygel®	Membrane Filtration
1 in 100	CRW	D	4	4	4	4	4
2 in 100	CRW	D	4	4	4	4	4
5 in 100	CRW	D	4	4	4	4	4
10 in 100	CRW	D	4	4	4	4	4
15 in 100	CRW	D	4	4	4	4	4
25 in 100	CRW	D	4	4	4	4	4
50 in 100	CRW	D	4	4	4	4	4
75 in 100	CRW	D	4	4	4	4	4
undiluted	CRW	D	4	4	4	4	4
Blank	n/a	n/a	2	2	2	2	2
Duplicate	CRW	D	2	2	2	2	2
		TOTAL	**40**	**40**	**40**	**40**	**40**

n/a: not applicable
1: Dilution fractions presented should be read as 1 mL of sample in 100 mL of deionized water.
2: Source for all samples is the Charles River Water.
3: Since the Charles River is surface water, it is a "Doubtful" source as per the Capiz PHO definition.

5.3. 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 H_2S and Easygel® tests were suggested as potential complementary tests to the EC-Kit, to be verified during the Capiz Province water quality testing program.

5.3.1. H_2S test

The H_2S test using the original M1 medium is a well-known, simple and low-cost P/A test, developed by Manja, Maurya, and Rao (1982). The test identifies the presence of H_2S-

producing bacteria, associated with fecal contamination in a volume of water, which has been shown to correlate with the presence of fecal contamination.

Venkobachar, Kumar, Talreja, Kumar, and Iyengar (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 H_2S test (Pillai, Mathew, Gibbs, & Ho, 1999).

The M1 and M2 media were used during the preliminary laboratory testing undertaken at MIT (November 2009); and the M2 test medium was used throughout the water quality testing program in Capiz Province in January 2010 for all sample sources: from open dug wells (Doubtful source) to piped, chlorinated tap water (Level 3 source). Indeed, since the H_2S test reagent includes a chlorine-neutralizing compound (sodium thiosulfate), the H_2S test is a suitable microbiological test for chlorinated water supplies.

The M2 test medium was also used during the MIT laboratory testing in April 2010. Section 1.6.2. provides more information on making the H_2S paper strips for this study.

Another H_2S test used in this study is the industry-made HACH Pathoscreen™. This test uses a powder-form, dehydrated H_2S test reagent, suitable for a 20-mL sample volume.

Appendix A gives a step-by-step procedure on how to make the H_2S reagents (M1 and M2) used in this study and how to conduct tests and interpret results.

5.3.2. Easygel®

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

The Easygel® medium contains a sugar linked to a dye which, when acted on by the coliform-produced enzyme β-galactosidase turns the colony a pink color. Similarly, a second sugar linked to a different dye, which when acted on by the *E.coli*-produced enzyme β-glucuronidase turns the colony a blue-green color. This allows the count of total coliform colonies: pink colonies, and of *E.coli* colonies: purple (pink + blue) colonies (Micrology Laboratories, 2008).

One of the main advantages of the Easygel® is that it serves as an agar replacement. Agar is difficult to prepare, requires specific reagents and equipment, and preparation is both labor- 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 at MIT.

An added benefit of the Easygel® 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.

One of the drawbacks of Easygel® is that the media must be stored in the freezer before it is used. However, (Micrology Laboratories, 2009) states that the media bottle can be left out at ambient temperature up to one month prior to use.

Appendix B provides information on how to test and interpret Easygel® tests.

5.3.3. EC-Kit

A portable microbiology laboratory testing kit was initially developed by Robert Metcalf, PhD, one of the original founders of Solar Cookers International, and Professor of Microbiology at California State University at Sacramento. Susan Murcott then modified the testing kit to include a waist belt incubator, which incubates water samples using body temperature alone. The waist belt incubator serves as a cheaper, portable, and more convenient alternative to 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 simple name "EC-Kit."

The EC-Kit contains two complementary tests for *E.coli*: the Colilert 10-mL P/A test, and 3M™'s Petrifilm™ test. The Colilert P/A test is 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 lower detection level equivalent to 10 MPN/100 mL, whereas Quanti-Tray® has a lower detection limit of 1 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.

In addition to the two tests, the kit also includes 100-mL sterile sample bags, individually wrapped, sterile 3.5-mL pipettes, an ultraviolet light with batteries, cardboard squares with rubber bands, and a waist belt incubator.

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 that bacterial incubation are all performed using the waist-belt incubator, so it is completely portable.

One of the drawbacks of the Petrifilm™ is that, although it is a more efficient water quality testing method than membrane filtration, for example, the open package of unused Petrifilms™ must be stored in the refrigerator.

Chuang (2010) has verified the EC-Kit against Quanti-Tray® through wide-scale testing both in Capiz Province, and at the MIT laboratory. Only once the EC-Kit has been tested and compared to Quanti-Tray® can its results be deemed "valid."

Appendix C provides information on how to test and interpret EC-Kit tests.

5.3.4. 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 MPN.

The enzyme substrate test uses hydrolysable substrates for the detection of both total coliform and *E.coli* enzymes. When the enzyme technique is used, the total coliform group is defined as all bacteria possessing the enzyme β-D-galactosidase, which adheres to the chromogenic substrate, resulting in release of the chromogen (the sample changes color and becomes yellow). *E.coli* bacteria are defined as bacteria giving a positive total coliform response and possessing the enzyme β-glucuronidase, which adheres to a fluorogenic substrate, resulting in the release of the fluorogen (the sample fluoresces) (APHA, AWWA, WPCF, 2007).

The MPN method is an important quantitative tool in estimating the microbial population present in a given water sample. It uses multiple qualitative (P/A) data points (for Quanti-Tray®, the number of positive wells out of 50 wells and for Quanti-Tray®/2000, the number of positive large wells out of 49 and the number of positive small wells out of 24) to generate a maximum probability coliform count per 100 mL value, given by a standard MPN table. 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.

The Quanti-Tray® provides bacterial counts (of total coliform and *E.coli*) as low as 1 MPN/100mL and up to 200.5 MPN/100 mL of sample, whereas the Quanti-Tray®/2000 provides a bacterial count as low as 1 MPN/100mL and up to 2419 MPN/100 mL. Both tests have a better 95% confidence limit than multiple tube fermentation (IDEXX, 2010b).

Looking back, 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.

The Quanti-Tray® is easy-to-use, rapid and accurate, and has been approved by the US EPA, and over 35 countries for drinking, source/surface, ground, and waste- waters (IDEXX, 2010a). 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 are particularly expensive ($21/test in Capiz), especially in developing countries.

5.3.5. Membrane Filtration

The Standard Total Coliform and Fecal Coliform Membrane Filter Procedures (Standard Methods 9222a and 9222b, respectively) is an enumerative testing procedure that yields a coliform count (total, fecal or *E.coli*) per sample volume (from 100 mL to dilutions down to volumes as low as necessary).

The Membrane Filter Procedure uses pre-sterilized, 0.45 micron membrane filters such that there is complete retention of coliform bacteria; pre-sterilized absorbent pads; pre-sterilized glass culture dishes and filtration units; and the membrane filtration, Endo-type culture medium (m-ColiBlue24®). The filter and medium used in this study allowed the detection of both total coliform and *E.coli* colonies.

Membrane filtration is fairly expensive, and the testing procedure is more complex than the other microbiological tests presented here. However, it has been the USEPA-set standard for microbiological testing of drinking water in the United States and other countries. Given the membrane filtration's complexity and cost, it was not selected as a potential field-based test for water quality testing in Capiz Province.

6. Results

6.1. Overall Test Results

The test results presented in this chapter include results obtained from water quality testing in Capiz Province and at the MIT laboratory. In the following section, results are initially presented separately, although the test results obtained from Capiz and MIT will be combined later for statistical analyses.

6.1.1. Test Results from Capiz Province

The complete tests results from water samples collected in Capiz Province in January 2010, and tested using Quanti-Tray®, EC-Kit, H_2S test and Easygel® are presented in Appendix G. The test results presented here include both the *E.coli* and total coliform counts for Easygel®, EC-Kit, and Quanti-Tray®.

On average, for all tests performed (H_2S tests, Easygel®, Quanti-Tray® and EC-Kit), approximately 55% of all water samples tested were positive for fecal contamination. The H_2S test identified 61%, 66%, 74% and 60% of samples to be positive for H_2S-producing bacteria, with sample volumes of 10 mL (n=163), 20 mL (n=163), 100 mL (n=162) and the 20-mL HACH test (n=163), respectively; Easygel® (n=43) identified 46% of samples as positive for *E.coli*; Quanti-Tray® (n=178) identified 62% of water samples as being positive for *E.coli*; EC-Kit identified 54% (Colilert, n=178) and 38% (Petri-Film, n=178) as positive for *E.coli*. Figure 6-1 presents a graph of the percentage of positive results obtained per microbiological test in Capiz Province.

6.1.2. Test Results from MIT Laboratory Studies

The complete tests results from water samples collected from the Charles River on April 4, 2010 and tested using Quanti-Tray®, EC-Kit, H_2S test, Easygel® and membrane filtration are presented in Appendix G. Dilutions of the Charles River were prepared in the laboratory: 1, 2, 5, 10, 15, 25, 50, and 75 mL of Charles River in 100 mL of deionized water. Undiluted Charles River water was also tested. Four water samples were tested per dilution level, totaling 32 samples, in addition to four undiluted Charles River water samples, two duplicates and two blanks.

In general, for all tests performed, the vast majority of Charles River water dilutions tested were positive for *E.coli*, and, as expected, all blanks were negative for *E.coli.* The H_2S test identified 80%, 88%, 95% and 80% of samples to be positive for H_2S-producing bacteria with sample volumes of 10 mL (n=40), 20 mL (n=40), 100 mL (n=40) and the 20-mL HACH test (n=40), respectively; Easygel® (n=39) identified 77% of samples as positive for *E.coli*; Quanti-Tray® (n=40) identified 95% of water samples as being positive for *E.coli*; EC-Kit identified 95% (Colilert, n=40) and 53% (Petrifilm, n=38) as positive for *E.coli*; and membrane filtration (n=40) identified 95% of samples as positive for *E.coli.* Figure 6-1 presents a graph of the percentage of positive results obtained per microbiological test at the MIT laboratory.

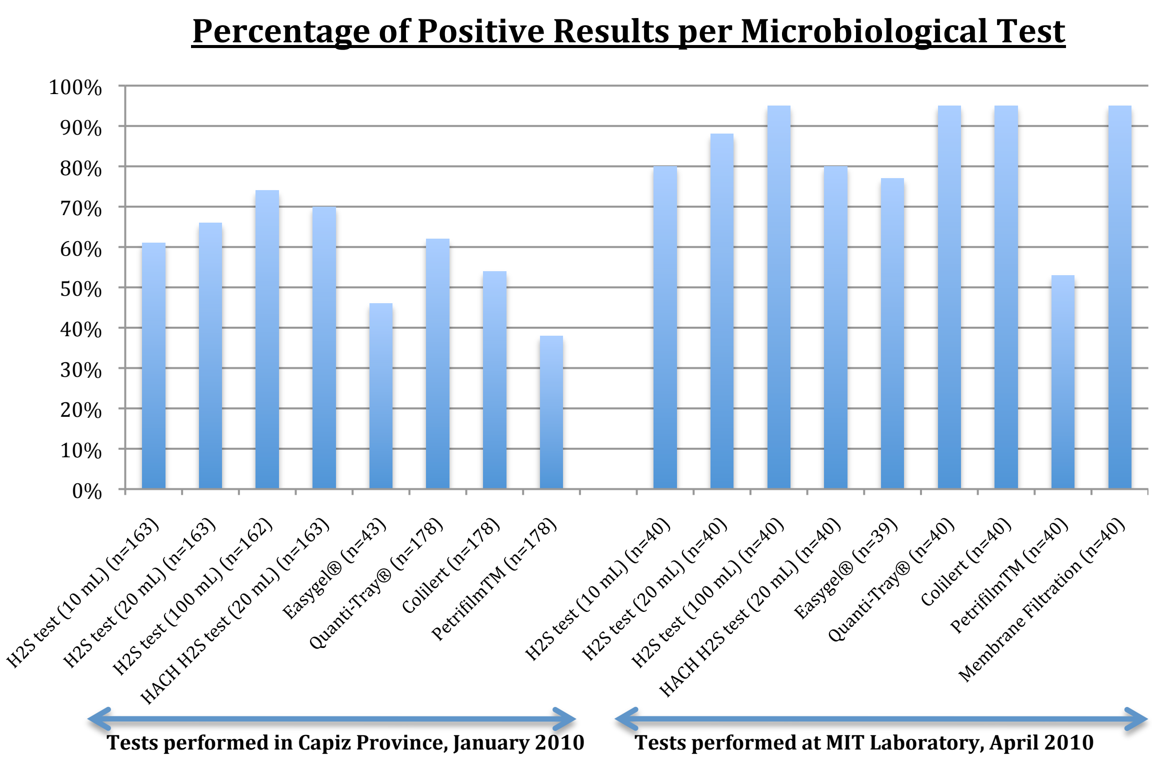

Figure 6-1. Percentage of Positive Results per Microbiological Test.

6.2. EC-Kit Errors in Capiz

6.2.1. Potential Sources of Error in Performance of EC-Kit Tests in Capiz

As mentioned earlier, the PHO drinking water quality testing program from December 2009 to March 2010 was the first time that drinking water quality had ever been measured throughout the province. As such, it is important to mention that the SI's who were conducting the field sampling and EC-Kit tests had no prior sampling or water quality laboratory experience. They all attended a training session in May 2009, conducted by employees of the NGO "A Single Drop", who had themselves been introduced to the EC-Kit by Susan Murcott, and trained in using the EC-Kit test by Robert Metcalf. During this training, it is assumed that participants were taught the proper sampling and testing methodology. When the MIT team began sampling with the municipal SI's, they witnessed sampling, testing and reading errors. The most common errors are summarized in Table 6-1.

Table 6-1. Most Common Errors committed by SI's in the performance of EC-Kit test methods.

Sampling Errors	Testing Errors	Reading Errors
▪ Inability to recognize the water sources to be sampled (for example, jetmatic pump from shallow well pump) ▪ Not letting the water from a tap/pump run for more than 1 minute prior to sampling. ▪ Placing fingers inside the sterile sampling bag. ▪ Inserting the spout of the tap/well into the sample bag. ▪ Forgetting to bring a cooler with ice packs to the field.	▪ Not washing hands or wiping down surface prior to conducting the tests. ▪ Pipetting inaccurate volumes onto the Petrifilm™ ▪ Labeling the Petrifilm™ sample on the clear plastic above the Petrifilm™. ▪ Not wearing the incubator belt for 24 hours, continuously.	▪ Colilert: Misinterpreting fluorescence in samples. ▪ Petrifilm™: Counting blue/red colonies without gas bubbles as *E.coli* and total coliform colonies. ▪ Petrifilm™: Not counting colonies if more than 10.

6.2.2. Steps Taken to Correct EC-Kit Errors in Capiz

In mid-January 2010, these errors were reported to Jane Delos Reyes of the Capiz PHO. At that point, all the EC-Kit tests (Colilert and Petrifilm™) performed since the beginning of the water quality testing program were recalled and verified by the MIT team and Jane Delos Reyes. Appropriate corrections, based on the recalled test readings, were made to the results data set. Finally, to avoid further mistakes, a new EC-Kit training session for municipal SI's took place in February 2010.

6.3. Statistical Analysis of Results

The statistical analyses of water quality test results, collected in Capiz Province and at MIT, were used to determine the accuracy of different field-based, microbiological tests: H_2S test, Easygel® and EC-Kit (Colilert and Petrifilm™) through comparison with two standard methods: Quanti-Tray® and membrane filtration.

The data presented here is a compilation of the data collected in Capiz Province in January and the data obtained through MIT laboratory testing of the Charles River. The statistical analysis for Capiz Province results compares the field-based microbiological tests (H_2S test, Easygel® and EC-Kit) to Quanti-Tray®. The statistical analysis for the MIT laboratory test results compares the field-based microbiological tests (H_2S test, Easygel® and EC-Kit) to Quanti-Tray® and membrane filtration. All statistical results presented here were analyzed using the STATA® Release II software.

6.3.1. Statistical Analyses: Background and Definition

One of the main difficulties with comparing the low-cost microbiological tests to Standard Methods tests is that these tests give different outputs: some are qualitative (i.e. P/A tests) and some are enumerative (i.e. yield numerical results in CFU/mL or MPN/mL). Therefore, two sets of statistical analyses were performed. P/A tests, namely the H_2S and Colilert tests, were analyzed using 2x2 contingency tables, analyzed using general statistical analyses (True Results (TR), False Positives (FP), False Negatives (FN), Positive Predictive Value (PPV) and Negative Predictive Value (NPV)) following a method described by Mack and Hewison (1988) and tested for statistical significance (chi-square test and Fisher's exact test). Those quantitative tests, namely Easygel® and Petrifilm™, were analyzed by using n x n contingency tables, and tested for statistical significance (chi-square test and Fisher's exact test) and by scatter plot. The enumerative tests were also analyzed using the same statistical analyses used to analyze the P/A tests. Finally, a combination of tests (P/A + enumerative) in addition to the EC-Kit, were set-up and analyzed based on general statistical analyses, Error, and the Proportional Reduction in Error. More information on each of these tests is provided below.

6.3.2. P/A Tests

6.3.2.1. 2x2 Contingency Tables

A 2x2 contingency table (Table 6-2) 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. Standard Method and New Test), 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.

Table 6-2. 2x2 Contingency Table.

	Standard Method	
New Test	*Presence*	*Absence*
Presence	a	b
Absence	c	d

6.3.2.2. General Statistical Analyses

When a New Test is being compared against a Standard Method, the percentage of TR's (*a*+*d*), FP's (*b*) and FN's (*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 negative, or to incorrectly flag a negative result when it should be positive.

Furthermore, for the 2x2 contingency table, we used four general correlation analyses (sensitivity, specificity, PPV and NPV), to determine the "goodness of fit" of the New Test to the Standard Method (Nair, Gibbs, & Mathew, 2001). It is important to note that these four correlations operate under the assumption that the Standard Method is in itself a perfect test that yields 100% true results.

True result

True result (TR) represents the percentage of samples tested by the New Test that yielded the same result as the Standard Method test (e.g. Absence and Absence).

$$TR = \frac{a+d}{a+b+c+d}$$

False positive

False positive (FP) represents the percentage of positive samples tested by the New Test that yielded a negative result as the Standard Method test.

$$FP = \frac{b}{a + b + c + d}$$

False negative

False negative (FN) represents the percentage of negative samples tested by the New Test that yielded a positive result as the Standard Method test.

$$FN = \frac{c}{a + b + c + d}$$

Sensitivity

Sensitivity is the ability of the New Test to determine a true positive result (Mack & Hewison, 1988).

$$Sensitivity = \frac{a}{a + c}$$

Specificity

Specificity is the ability of the New Test to determine a true negative result (Mack & Hewison, 1988).

$$Specificity = \frac{d}{b + d}$$

Positive Predictive Value (PPV)

PPV is the ability of a positive test (by the New Test) to predict the presence of a contaminant, *E.coli* in our case (Mack & Hewison, 1988).

$$PPV = \frac{a}{a + b}$$

Negative Predictive Value (NPV)

NPV is the ability of a negative test (by the New Test) to predict the absence of a contaminant, *E.coli* in our case (Mack & Hewison, 1988).

$$NPV = \frac{d}{c + d}$$

6.3.2.3. Statistical Significance

Statistical significance is a procedure for establishing the degree of confidence that one can have in making an inference from a sample to its parent population (Meier, Brudney, & Bohte, 2009). In other words, it tells you how sure you are (*p*-value) that the two variables you are comparing are related or not. The importance of the *p*-value is that it tells us exactly how significant the results are. The challenge in determining statistical significance lies in assessing the *p*-value: how small should the *p*-value be to be statistically significant. Table 6-3 lists some commonly used criteria for judging the significance of a *p*-value (Rosner, 2006).

Table 6-3. Guidelines for Assessing the Significance of a *p*-value (Rosner, 2006).

***p*-value (*p*)**	**Significance of *p*-value**
$0.01 \leq p < 0.05$	Results are *significant*.
$0.001 \leq p < 0.01$	Results are *highly significant*.
$p < 0.001$	Results are *very highly significant*.
$p > 0.05$	Results are considered *not statistically significant*.
$0.05 \leq p < 0.1$	There is a *trend toward statistical significance*.

The Chi-Square Test

The chi-square test is a procedure for evaluating the level of statistical significance attained by a bi-variate relationship in a cross-tabulation. The chi-square test assumes there is no relationship between the two variables (i.e. between the Standard Method and the New Test), in other words, that the respective tests are independent variables, and determines whether any apparent relation can be attributed to chance. The chi-square test involves three steps:

1. Expected frequencies are calculated for each cell in the 2x2 contingency table based on the assumption that the two variables are unrelated in the population.
2. The chi-square value ($\chi 2$) is calculated based on the difference between the expected and actual frequencies
3. The chi-square value is compared with a table of theoretical chi-square values and their corresponding p-values.

It is important to note that since the expected frequencies were calculated based on the assumption of no relationship, then the greater the difference between them (chi-square value) then the greater the departure from the null hypothesis (meaning there is no relationship) and the greater the association with an alternate hypothesis (meaning that there is a relationship).

The chi-square test can only be computed for a 2x2 contingency table for which all cell values are greater than or equal to 5. For contingency tables that do not satisfy this criterion, Fisher's exact test is used.

Fisher's Exact Test

Fisher's exact test gives exact results for any 2x2 contingency table, but since it is more complicated to calculate, it is only used for tables with small cell values (less than 5). The *p*-value determined from Fisher's exact test is very similar to the chi-square test (Rosner, 2006).

6.3.2.4. Error and Proportional Reduction in Error, λ

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 $Error_1$) and then adding another piece of information (in this case, a New Test) to obtain a test result that will hopefully yield a better prediction (with corresponding $Error_2$). The formula for λ is provided below.

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

In this case, the initial assumption was that U.N.-designated unimproved water sources (or Doubtful sources in the Philippines) were all contaminated (High/Very High Risk Level or Presence of contaminant), and that U.N.-designated improved water sources (or Levels 1 through 3 in the Philippines) were all safe (Conformity/Low Risk Level or Absence of contaminant). An example of two 2x2 contingency tables (Table 6-4 for unimproved water sources and Table 6-5 for improved water sources) obtained from the initial assumptions are provided below.

Table 6-4. Example of a 2x2 Contingency Table from an Initial Prediction based on an Unimproved Source.

Prediction	Standard Method: *Presence*	Standard Method: *Absence*
Presence	38	3
Absence	0	0

Table 6-5. Example of a 2x2 Contingency Table from an Initial Prediction Based on an Improved Source.

Prediction	Standard Method: *Presence*	Standard Method: *Absence*
Presence	0	0
Absence	66	60

From this, the error associated with the initial prediction ($Error_1$) can be computed and compared to $Error_2$ associated with a single field-based test, or with a combination of field-based tests.

Since the initial prediction requires a known U.N. classification of drinking water (improved or unimproved), the Charles River dilution samples (collected in April 2010) were not included in this statistical analysis (Error and λ). Only the Capiz water samples were subject to this analyses, and could only be compared to the only Standard Methods test used in Capiz: Quanti-Tray®.

6.3.3. Enumerative Tests

In addition to the statistical analyses listed above, enumerative tests were also analyzed based on the following.

6.3.3.1. Scatter Plots

Scatter plots (Standard Method vs. New Test) were made for all enumerative tests (Easygel®, Petrifilm, against Quanti-Tray® and membrane filtration). If the graph slopes upward, then there is a positive correlation between the two variables; conversely, if the graph slopes downward, then there is a negative correlation between the two variables.

The scatter plots also included vertical and horizontal lines that delineate the WHO risk levels (from Conformity to Very High, depending on a given test's detection limit) so as to visually appraise the New Test and Standard Method correlation in terms of risk levels.

6.3.3.2. n x n Contingency Tables

3x3 contingency tables serve the same purpose as 2x2 contingency tables. However, since enumerative tests give more information on the degree of *E.coli* contamination, then a higher degree (n x n) contingency 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).

6.3.3.3. Statistical significance

The tests for statistical significance for a n x n contingency table are identical to those for a 2x2 contingency table: chi-square test and Fisher's exact test.

6.4. H_2S Test

6.4.1. Compared to Quanti-Tray®

The 2x2 contingency table for all new field-based tests compared to Quanti-Tray® test results are presented in Appendix H. The corresponding TR, FP, FN, Sensitivity, Specificity, PPV and NPV values are presented in Table 6-6 below for Capiz and Cambridge samples combined.

The H_2S medium was used to test 163 water samples in Capiz Province from different sources (springs, protected 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, MA: 38 from the Charles River, and 2 de-ionized water samples.

When comparing the H_2S laboratory-made reagents, the 20-mL test gave slightly more true results than the 100-mL and 10-mL tests. 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 FN's was highest for the 10-mL test (11%) and lowest for the 100-mL test (4%).

Table 6-6. Percentage of True and False Results, Sensitivity, Specificity, PPV and NPV Results for New, Field-Based Tests Compared to Quanti-Tray® for Capiz and Cambridge Samples.

		True Results	False Positives	False Negatives	Sensitivity	Specificity	PPV	NPV
Lab-made reagents	10-mL H_2S test (n=203)	80	9	11	84	72	85	69
	20-mL H_2S test (n=203)	84	10	6	91	71	86	80
	100-mL H_2S test (n=203)	80	16	4	94	53	80	82
Manufactured and purchased	HACH test (n=203)	79	9	12	82	72	85	68

In general, it was noted that as the sample volume of the H_2S test increased, sensitivity also increased from 84% for the 10-mL test to 94% for the 100-mL test, which means that the higher volume test can detect more true positives; whereas specificity decreased dramatically from 72% for the 10-mL test to 53% for the 100-mL test. Also, the PPV value for the 10- and 20-mL tests were similar at 85% to 86%, but was much smaller for the 100-mL test (53%); in other words, a positive test is no longer directly synonymous with presence of fecal contamination. Finally, NPV increased with increasing sample volume from 69% for the 10-mL test to 82% for the 100-mL test; so a negative test becomes more likely to reflect absence of fecal contamination.

The 20-mL HACH PathoScreen™ test had results that were very similar to the 10-mL H_2S test, although it still proved to be the least accurate of all the H_2S tests: it had the lowest percentage of true results (79%) and although its percentage of FP's was low (9%), it had the highest percentage of FN's (12%), and lowest sensitivity and NPV values: 82% and 68%, respectively.

The high percentage of FP results (12%) and low specificity of the 100-mL test is probably due to the H_2S test detecting H_2S that may not come from fecal bacteria. In groundwater in particular, there is the strong possibility of H_2S being present due to natural geohydrological sources and to anthropogenic impacts other than fecal contamination, both of which would lead to FP results (Sobsey & Pfaender, 2002). Indeed, this hypothesis was confirmed during the water quality testing of the Charles River, where all samples that were positive for *E.coli* by the H_2S tests were also positive for *E.coli* by Quanti-Tray®.

This phenomenon is especially important in this study since most drinking water samples (136 samples) were groundwater collected from wells and spring sources. Furthermore, it has been shown that the H_2S test detects bacteria other than coliforms that are associated with fecal contamination, such as *Clostridium perfringens*, which is one of the most resistant indicators of fecal contamination. Therefore it is possible that the H_2S 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 FN's, in other words not detecting fecal contamination when it is present. The percentage of FN's was relatively low for the 10-mL sample (11%), but was reduced by almost half in the 100-mL sample (4%). The higher percentage of FP's versus FN's in the 20- and 100-mL tests is favorable because it errs on the side of caution. For the 10-mL H_2S test and HACH test, the percentage of FN's was higher than the percentage of FP's.

According to the (WHO, 2008), *E.coli* must not be detectable in any 100-mL sample of water directly intended for drinking. Therefore it is important to determine the lower detection limit of the H_2S tests to ensure that the test yields a positive result if a water sample has an *E.coli* concentration greater than 1 CFU/100 mL.

Table 6-7 presents the percentage of FN's results obtained per WHO Risk Level. These values were obtained by identifying the samples that were negative for the H_2S test but positive for Quanti-Tray®, and determining, according to their Quanti-Tray® enumerative test result, what WHO Risk Level the water sample fell into. From Table 6-7, it can be noted that the 10-mL H_2S test had a large percentage of FN's in the Intermediate Risk Level (16%); these FN's were for samples with an *E.coli* concentration less than 45.3 MPN/100 mL. Similarly, the HACH test failed to detect approximately 7% of the samples in the Intermediate Risk Level; these FN's were for samples with an *E.coli* concentration less than 30.6 MPN/100 mL. The 20-mL H_2S test had some difficulty detecting *E.coli* in the Intermediate Risk Level, although the vast majority of the FN's in this range were for samples with *E.coli* concentrations less than 45 MPN/100 mL. Also, it is important to note that all H_2S tests, with the exception of the 100-mL test, failed to detect the presence of *E.coli* in the High/Very High Risk Level. This sample had an *E.coli* concentration greater than >201 MPN/100 mL. On the other hand, the 100-mL H_2S test had no FN's for samples in the Intermediate and High/Very High Risk Levels. The FN's in the Conformity/Low Risk Level were for samples with *E.coli* concentrations less than 7.5 MPN/100 mL.

Table 6-7. Percentage of False Negatives per WHO Risk Level per H_2S Test for Capiz and Cambridge Samples.

	Percentage of False Negative Results (%)			
WHO Risk Level (CFU/100mL)	**10-mL H_2S test**	**20-mL H_2S test**	**100-mL H_2S test**	**20-mL HACH test**
Conformity/Low (<10) (n=98)	13	5	8	14
Intermediate (10-100) (n=44)	16	7	0	7
High/Very High (>100) (n=60)	3	2	0	2

6.4.1.1. Chi-Square and Fisher's Exact Tests

Table 6-8 lists the values obtained from the Chi-square (χ^2 and p) and Fisher's exact test for H_2S tests compared to Quanti-Tray® for Capiz and Cambridge samples. These statistical results were calculated using Stata® Release II software.

Table 6-8. Chi-Square value, *p*-value, Fisher's Exact Test Probability and Statistical Significance for H_2S Tests Compared to Quanti-Tray® for Capiz and Cambridge Samples.

	Chi-square value	***p*-value**	**Fisher's exact test probability**	**Statistical significance**
10-mL H_2S test (n=203)	62.5077	0.000	0.000	Very highly significant
20-mL H_2S test (n=203)	83.4533	0.000	0.000	Very highly significant
100-mL H_2S test (n=202)	58.4138	0.000	0.000	Very highly significant
HACH test (n=203)	58.5255	0.000	0.000	Very highly significant

These results show that there is a very significant statistical relationship between the H_2S tests and Quanti-Tray®.

6.4.1.2. Error and Proportional Reduction in Error, λ

The tables used in calculating the error and proportional reduction in error, λ, for improved and unimproved water sources are presented in Appendix I. 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. The actual error and λ values are presented in Table 6-9.

Table 6-9. Error and Proportional Reduction in Error for H_2S tests for Capiz samples.

Test	Unimproved Sources			Improved Sources		
	Error	λ	n[1]	Error	λ	n[2]
10-mL H_2S test	9.1%	0.0%	33	24.6%	51.5%	130
20-mL H_2S test	9.1%	0.0%	33	20.0%	60.6%	130
100-mL H_2S 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 H_2S test.
[2]: Sample size for improved sources tested for given H_2S test.

It is interesting to note that errors for the H_2S test were greater for improved sources than for unimproved sources. For unimproved sources, the addition of the 10-, 20-, and 100-mL H_2S test did not change the error (λ=0%), however the addition of the HACH test *increased* the error by 133%. Therefore as a single test for unimproved sources, the laboratory-made H_2S test is no better than simply predicting that all unimproved sources are contaminated. For improved sources, the addition of all H_2S tests (laboratory made and HACH test) reduced our error on average by 52.7%, with an average error 24.1%.

The numbers presented in Table 6-9 are not identical to those presented in Section 5.4.2.1, since the sample size is different (Capiz samples only), and since the test results have been divided between unimproved and improved sources.

6.4.2. Compared to Membrane Filtration

6.4.2.1. 2x2 Contingency Table

The 2x2 contingency table for the 10-, 20-, 100-mL H_2S tests and HACH test results compared to membrane filtration are presented in Appendix H. The corresponding TR, FP, FN, Sensitivity, Specificity, PPV and NPV values are presented in Table 6-10 below for Cambridge samples only.

In general, the same trend is noted here as in the comparison with Quanti-Tray® explained above. As the sample size for the H_2S test increases from 10- to 100-mL, the percentage of FN's decreases, sensitivity levels and NPV values increase. Also, HACH test values are identical to the H_2S 10-mL test values.

Table 6-10. Percentage of True and False Results, and Sensitivity, Specificity, PPV and NPV Results for H_2S Tests Compared to Membrane Filtration for Cambridge samples.

	True Results	False Positives	False Negatives	Sensitivity	Specificity	PPV	NPV
10-mL H_2S test (n=40)	85	0	15	84	100	100	25
20-mL H_2S test (n=40)	93	0	8	92	100	100	40
100-mL H_2S test (n=40)	100	0	0	100	100	100	100
HACH test (n=40)	85	0	15	84	100	100	25

Something to note is that in this case, unlike the comparison with Quanti-Tray®, the percentage of true results here is greater for the 100-mL H_2S test than for the 20-mL H_2S test. The 100% results for the specificity and PPV criteria is probably due to high level of contamination present in Charles River (even in a 1 in 100 dilution), whereas samples collected in Capiz did not all have this high level of contamination.

6.4.2.2. Chi-Square and Fisher's Exact Tests

Table 6-11 lists the values obtained from the Chi-square (χ^2 and p) and Fisher's exact test for H_2S tests compared to membrane filtration for Cambridge samples only.

Some cells are marked "n/a" because some cells in the contingency table contained values less than 5, therefore the chi-square test was not applicable. Instead, only Fisher's exact test was used to determine statistical significance. Most of the conclusions for statistical significance reached here in the comparison of the H_2S tests with membrane filtration is that there is a "trend toward statistical significance" which is probably due to the small sample size (n=40) used in this comparison. In general, a larger sample size would confirm that there is a relationship between membrane filtration and H_2S tests, although this has not been proven here.

Table 6-11. Chi-Square Value, *p*-value, Fisher's Exact Test Probability and Statistical Significance for H_2S Tests Compared to Membrane Filtration for Cambridge Samples.

	Chi-square value	*p*-value	Fisher's exact test probability	Statistical significance
10-mL H_2S test (n=40)	n/a	n/a	0.036	Trend toward statistical significance
20-mL H_2S test (n=40)	n/a	n/a	0.013	Trend toward statistical significance
100-mL H_2S test (n=40)	n/a	n/a	0.001	Highly significant
HACH test (n=40)	n/a	n/a	0.036	Trend toward statistical significance

n/a: not applicable

6.5. Easygel®

6.5.1. Compared to Quanti-Tray®

6.5.1.1. 2x2 Contingency Table

The 2x2 contingency table for the Easygel® test compared to Quanti-Tray® test results is presented in Appendix H. The corresponding TR, FP, FN, Sensitivity, Specificity, PPV and NPV values are presented in Table 6-12 below for Capiz and Cambridge samples combined.

Table 6-12. Percentage of True and False Results, and Sensitivity, Specificity, PPV and NPV Results for Easygel® Test Compared to Quanti-Tray® for Capiz and Cambridge Samples.

	True Results	False Positives	False Negatives	Sensitivity	Specificity	PPV	NPV
Easygel® (n=83)	81	1	17	78	94	98	55

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

The Easygel® test had a relatively high percentage of TR's (81%), few FP's and a high proportion of FN's. The Sensitivity and NPV values for Easygel® were relatively low (78% and 55%, respectively), which means that the Easygel® test is not a particularly good indicator of the presence of contamination, and a negative Easygel® test result is sometimes (45% of the time) not synonymous with *E.coli* contamination. However, the Easygel® test boasts high Specificity and PPV values, which means that it is a particularly good indicator of the absence of contamination, and that a positive test result is usually indicative of the *E.coli* presence.

According to the (WHO, 2008), *E.coli* must not be detectable in any 100-mL sample of water directly intended for drinking. Therefore it is important to determine the lower detection limit of the Easygel® to ensure that the test yields a positive result if a water sample has an *E.coli* concentration greater than 1 CFU/100 mL.

Table 6-13 presents the percentage of FN results obtained per WHO Risk Level. These values were obtained by identifying the samples that were negative for the Easygel® test but positive for Quanti-Tray®, and determining, according to their Quanti-Tray® enumerative test result, what WHO Risk Level the water sample fell into.

From Table 6-13, it can be noted that the Easygel® test yields a large percentage of FN's in the Intermediate Risk Level (10%), although it must be noted that these values were determined with a relatively small sample size (n=20). FN's in the Intermediate Risk Level were obtained for samples with an *E.coli* concentration less than 34.4 MPN/100 mL.

Table 6-13. Percentage of False Negatives per WHO Risk Level for the Easygel® Test for Capiz and Cambridge Samples.

WHO Risk Level (CFU/100mL)	False Negative Results for the Easygel® test (%)
Conformity/Low (<10) (n=37)	11
Intermediate (10-100) (n=20)	10
High/Very High (>100) (n=24)	0

6.5.1.2. Chi-Square and Fisher's Exact Tests of 2x2 Contingency Table

Table 6-14 lists the values obtained from the Chi-square (χ^2 and p) and Fisher's exact test for the 2x2 contingency table Easygel® compared to Quanti-Tray® for Capiz and Cambridge samples. These statistical results were calculated using Stata® Release II software.

Table 6-14. Chi-Square Value, *p*-value, Fisher's Exact Test Probability and Statistical Significance for the 2x2 Contingency Table for the Easygel® Test Compared to Quanti-Tray® for Capiz and Cambridge Samples.

	Chi-square value	*p*-value	Fisher's exact test probability	Statistical significance
Easygel® test (n=83)	30.91065	0.000	0.000	Very highly significant

These results show that there is a very significant statistical relationship between the Easygel® test and Quanti-Tray®.

6.5.1.3. Error and Proportional Reduction in Error, λ

The tables used in calculating the error and proportional reduction in error, λ, for improved and unimproved water sources are presented in Appendix I. 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. The actual error and λ values are presented in Table 6-15.

Table 6-15. Error and Proportional Reduction in Error for Easygel® Test for Capiz Samples.

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.

For unimproved sources, the addition of Easygel® did not reduce our error, but in fact increased it (λ = -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 51.7%, with an error of 24.6%. Fisher's exact test on the contingency table for unimproved and improved sources in Capiz showed that these results are not statistically significant, due to their small sample size.

The numbers presented in Table 6-15 are not identical to those presented in Section 5.5.1.1, since the sample size is different (Capiz samples only), and since the test results have been divided between unimproved and improved sources.

6.5.1.4. 3x3 Contingency Table

The 3x3 contingency table for the Easygel® test compared to Quanti-Tray® test results is presented below in Table 6-16. This table presents the Easygel® and Quanti-Tray® test results broken down into three categories: the WHO Risk Levels (Conformity/Low, Intermediate and High/Very High) for Capiz and Cambridge samples.

Table 6-16. 3x3 Contingency Table for the Easygel® Compared to Quanti-Tray® for 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/5mL.

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 3x3 contingency 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 6-16). 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 caution as it can result in the rejection of water that may be safe to drink. This option is much better than misclassifying water that is not safe to drink as water that is.

6.5.1.5. Chi-Square and Fisher's Exact Tests of 3x3 Contingency Table

Table 6-17 lists the values obtained from the Chi-square (χ^2 and p) and Fisher's exact test for the 3x3 contingency table for Capiz and Cambridge samples. These statistical results were calculated using Stata® Release II software.

Table 6-17. Chi-Square Value, *p*-value, Fisher's Exact Test Probability and Statistical Significance for the 3x3 Contingency Table for the Easygel® Test Compared to Quanti-Tray® for Capiz and Cambridge Samples.

	Chi-square value	***p*-value**	**Fisher's exact test probability**	**Statistical significance**
Easygel® test (n=83)	50.1101	0.000	0.000	Very highly significant

These results show that there is a very significant statistical between the Easygel® test and Quanti-Tray®.

6.5.1.6. Scatter Plot

Figure 6-2 and presents the scatter plot of Easygel® test results against the Quanti-Tray® test results. The graph shows a positive correlation between the Easygel® and Quanti-Tray® test results. It is important to note that the Quanti-Tray® used for testing the Capiz water samples could only detect up to 200.5 MPN/100 mL. This explains the vertical scatter of samples at Quanti-Tray® = 200.5 MPN/100 mL for different Easygel® test results.

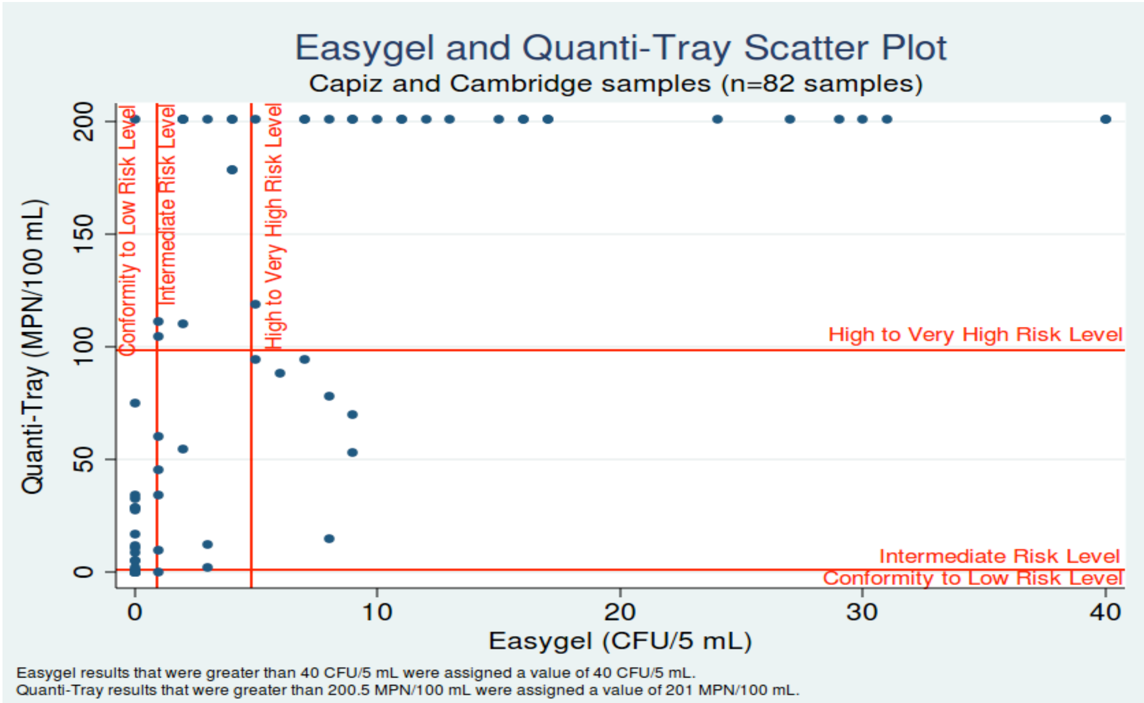

Figure 6-2. Easygel® vs. Quanti-Tray® Test Results with WHO Risk Levels for Capiz and Cambridge Samples.

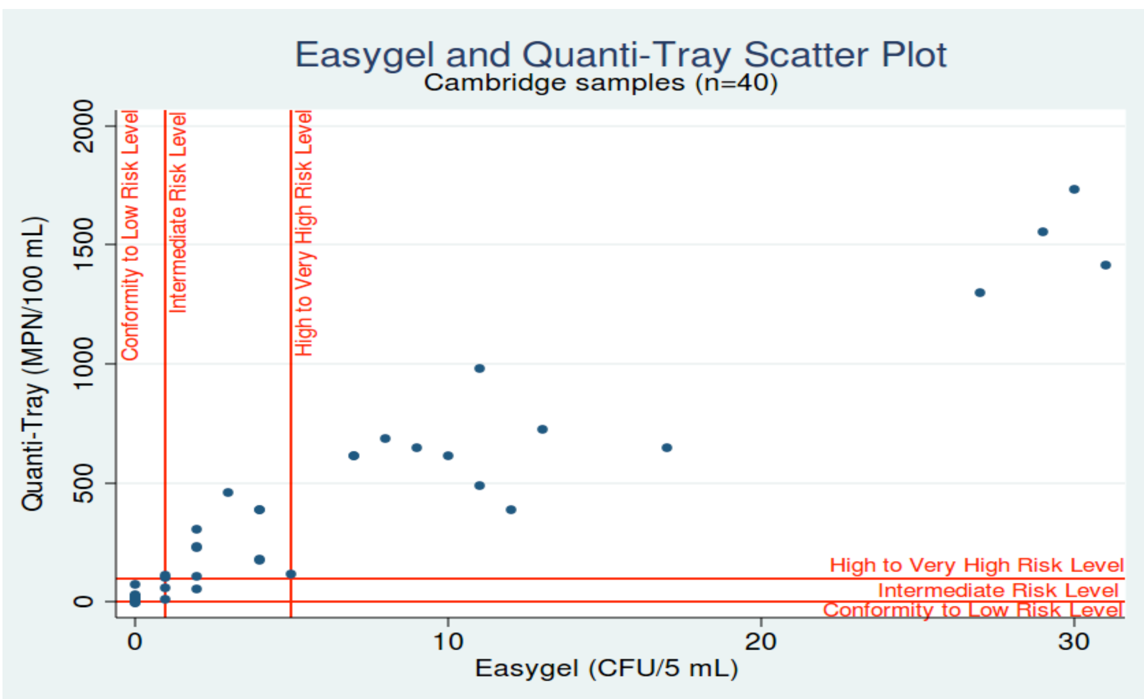

Figure 6-3. Easygel® vs. Quanti-Tray® Test Results with WHO risk levels for Cambridge Samples.

6.5.2. Compared to Membrane Filtration

6.5.2.1. 2x2 Contingency Table

The 2x2 contingency table for the Easygel® test compared to Quanti-Tray® test results is presented in Appendix H. The corresponding TR, FP, FN, Sensitivity, Specificity, PPV and NPV values are presented in Table -18 below for Cambridge samples only.

Table 6-18. Percentage of True and False Results, and Sensitivity, Specificity, PPV and NPV Results for Easygel® Test Compared to Membrane Filtration for Cambridge Samples Only.

	True Results	False Positives	False Negatives	Sensitivity	Specificity	PPV	NPV
Easygel® (n=40)	79	0	21	78	100	100	20

In general, the same trend is noted here as in the comparison with Quanti-Tray® explained above. The Easygel® test had a percentage of TR of 79%, a high percentage of FN's (21%), and 0% FP's. The Sensitivity and NPV values for Easygel® were low (78% and 20%, respectively), while the Specificity and PPV values were high (100%). These results also confirm the fact that the Easygel® test is not a particularly good indicator of the presence of contamination, but it is a much better indicator of the absence of contamination.

The 100% results for the specificity and PPV criteria is probably due to high level of contamination present in Charles River (even in a 1 in 100 dilution), whereas samples collected in Capiz did not all have this high level of contamination.

6.5.2.2. Chi-Square and Fisher's Exact Tests

Table 6-19 lists the values obtained from the Chi-square (χ^2 and p) and Fisher's exact test for Easygel® test compared to membrane filtration for Cambridge samples only. These statistical results were calculated using Stata® Release II software.

Table 6-19. Chi-Square Value, *p*-value, Fisher's Exact Test Probability and Statistical Significance for Easygel® Test Compared to Membrane Filtration for Cambridge Samples Only.

	Chi-square value	*p*-value	Fisher's exact test probability	Statistical significance
Easygel® test (n=40)	n/a	n/a	0.001	Very highly significant

n/a: not applicable

Some cells are marked “n/a” because some cells in the contingency table contained values less than 5, therefore the chi-square test was not applicable. Instead, only Fisher’s exact test was used to determine statistical significance. The result obtained from Fisher’s exact test shows that there is a very significant statistical relationship between the Easygel® test and membrane filtration.

6.6. Colilert

6.6.1. Compared to Quanti-Tray®

6.6.1.1. 2x2 Contingency Table

The 2x2 contingency table for the Easygel® test compared to Quanti-Tray® test results is presented in Appendix H. The corresponding TR, FP, FN, Sensitivity, Specificity, PPV and NPV values are presented in Table 6-20 below for Capiz and Cambridge samples combined.

Table 6-20. Percentage of True and False Results, and Sensitivity, Specificity, PPV and NPV Results for Colilert Test Compared to Quanti-Tray® for Capiz and Cambridge Samples.

	True Results	False Positives	False Negatives	Sensitivity	Specificity	PPV	NPV
Colilert (n=218)	83	5	11	83	84	92	70

The Colilert test (as a part of the EC-Kit) was used to test 178 water samples in Capiz Province from different sources (springs, protected 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, MA: 38 from the Charles River, and two de-ionized water samples.

The Colilert test had a relatively high percentage of TR’s (83%), few FP’s (5%) and a somewhat low proportion of FN’s (11%). The Sensitivity, Specificity and PPV values were relatively high (83%, 84% and 92%, respectively), which means that the Colilert test is a particularly good indicator of the presence of contamination. However, the Colilert test had a lower NPV value (70%), which means that a negative Colilert test is not always (30% of the time) synonymous with absence of *E.coli*.

According to the (WHO, 2008), *E.coli* must not be detectable in any 100-mL sample of water directly intended for drinking. Therefore it is important to determine the lower detection limit of the Colilert test to ensure that the test yields a positive result if a water sample has an *E.coli* concentration greater than 1 CFU/100 mL. Table 6-21 presents the percentage of FN results obtained per WHO Risk Level. These values were obtained by identifying the samples that were negative for the Colilert test but positive for Quanti-Tray®, and determining, according to their Quanti-Tray® enumerative test result, what WHO Risk Level the water sample fell into.

Table 6-21. Percentage of False Negatives per WHO Risk Level for the Colilert Test for Capiz and Cambridge Samples.

WHO Risk Level (CFU/100mL)	False Negative Results for the Colilert test (%)
Conformity/Low (<10) (n=104)	18
Intermediate (10-100) (n=48)	10
High/Very High (>100) (n=66)	1

From **Table 6-21**, it can be noted that the Colilert test yields a large percentage of FN's in the Low/Conformity and Intermediate Risk Levels (18% and 10%, respectively). FN's in the Intermediate Risk Level were obtained for samples with an *E.coli* concentration less than 62.4 MPN/100 mL.

6.6.1.2. Chi-Square and Fisher's Exact Tests

Table 6-22 lists the values obtained from the Chi-square (χ^2 and p) and Fisher's exact test for the 2x2 contingency table for Colilert compared to Quanti-Tray® for Capiz and Cambridge samples. These statistical results were calculated using Stata® Release II software.

Table 6-22. Chi-Square Value, *p*-value, Fisher's Exact Test Probability and Statistical Significance for the 2x2 Contingency Table for the Colilert Test Compared to Quanti-Tray® for Capiz and Cambridge Samples.

	Chi-square value	*p*-value	Fisher's exact test probability	Statistical significance
Colilert (n=218)	91.1323	0.000	0.000	Very highly significant

These results show that there is a very significant statistical between the Colilert test and Quanti-Tray®.

6.6.1.3. *Error and Proportional Reduction in Error, λ*

The tables used in calculating the error and proportional reduction in error, λ, for improved and unimproved water sources are presented in Appendix I. 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. The actual error and λ values are presented in Table 6-23.

Table 6-23. Error and Proportional Reduction in Error for Colilert Test for Capiz Samples.

Test	Unimproved Sources			Improved Sources		
	Error	λ	n[1]	Error	λ	n[2]
Colilert	4.88%	33.3%	41	22.2%	57.6%	126

[1]: Sample size for unimproved sources.
[2]: Sample size for improved sources.

It is interesting to note that errors for the Colilert test were greater for improved sources than for unimproved sources. For unimproved sources, the addition of the Colilert test decreased the error by a third, yielding a 5% error. Similarly, for improved sources, the Colilert test decreased the error by 58%, yielding an error of approximately 22%. Therefore, as a single test for unimproved and improved sources, the Colilert test is a useful, additional predictor of contamination.

The numbers presented in Table 6-26 are not identical to those presented in Section 5.6.1.1, since the sample size is different (Capiz samples only), and since the test results have been divided between unimproved and improved sources.

6.6.2. Compared to Membrane Filtration

6.6.2.1. *2x2 Contingency Table*

The 2x2 contingency table for the Colilert test compared to membrane filtration test results is presented in Appendix H. The corresponding TR, FP, FN, Sensitivity, Specificity, PPV and NPV values are presented in Table 6-24 below for Cambridge samples only.

Table 6-24. Percentage of True and False Results, and Sensitivity, Specificity, PPV and NPV Results for Colilert Test Compared to Membrane Filtration for Cambridge Samples Only.

	True Results	False Positives	False Negatives	Sensitivity	Specificity	PPV	NPV
Colilert (n=40)	100	0	0	100	100	100	100

In general, the same trend is noted here as in the comparison with Quanti-Tray® explained above. The Colilert test has a high percentage (100%) of TR, sensitivity, specificity and PPV, and a low percentage (0%) of FP and FN. However, unlike the Quanti-Tray® comparison, the Colilert as compared to membrane filtration has a high (100%) NPV value.

The 100% results for the true results, sensitivity, specificity, PPV and NPV criteria is probably due to high level of contamination present in Charles River (all Charles River samples was positive for presence of *E.coli*) were whereas samples collected in Capiz did not all have this high level of contamination (many had absence of *E.coli*).

6.6.2.2. Chi-Square and Fisher's Exact Tests

Table 6-25 lists the values obtained from the Chi-square (χ^2 and p) and Fisher's exact test for the 2x2 contingency table with WHO Risk Levels for Colilert compared to membrane filtration for Cambridge samples only. These statistical results were calculated using Stata® Release II software.

Table 6-25. Chi-Square Value, *p*-value, Fisher's Exact Test Probability and Statistical Significance for the 2x2 Contingency Table with WHO Risk Level for the Colilert Test Compared to Membrane Filtration for Cambridge Samples.

	Chi-square value	*p*-value	Fisher's exact test probability	Statistical significance
Colilert (n=40)	40.000	0.000	0.001	Very highly significant

These results show that there is a very significant statistical relationship between the Colilert test and membrane filtration.

6.7. Petrifilm™

6.7.1.1. Compared to Quanti-Tray®

6.7.1.2. 2x2 Contingency Table

The 2x2 contingency table for the Petrifilm™ test compared to Quanti-Tray® test results is presented in Appendix H. The corresponding TR, FP, FN, Sensitivity, Specificity, PPV and NPV values are presented in Table 6-26 below for Capiz and Cambridge samples.

Table 6-26. Percentage of True and False Results, and Sensitivity, Specificity, PPV and NPV Results for Petrifilm™ test compared to Quanti-Tray® for Capiz and Cambridge samples.

	True Results	False Positives	False Negatives	Sensitivity	Specificity	PPV	NPV
Petrifilm ™ **(n=218)**	67	3	30	55	91	93	49

The Petrifilm™ test was used to test 178 water samples in Capiz Province from different sources (springs, protected 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, MA: 38 from the Charles River, and two de-ionized water samples.

The Petrifilm™ test had a relatively low percentage of TR (67%), few FP's (3%) and a high proportion of FN's (30%). The Specificity and PPV values were high (91% and 93%, respectively), whereas the Sensitivity and NPV values were low (55% and 49%, respectively). This means that Petrifilm, like the Easygel® test, is a good indicator of the absence of *E.coli* contamination, but not a good indicator of the presence of *E.coli* contamination. Also, a positive result with the Petrifilm™ test is usually indicative of *E.coli* contamination, whereas a negative result is usually (51% of the time) not synonymous with absence of *E.coli* contamination.

According to the (WHO, 2008), *E.coli* must not be detectable in any 100-mL sample of water directly intended for drinking. Therefore it is important to determine the lower detection limit of the Petrifilm™ test to ensure that the test yields a positive result if a water sample has an *E.coli* concentration greater than 1 CFU/100 mL.

Table 6-27 presents the percentage of FN results obtained per WHO Risk Level. These values were obtained by identifying the samples that were negative for the Petrifilm™ test but positive for Quanti-Tray®, and determining, according to their Quanti-Tray® enumerative test result, what WHO Risk Level the water sample fell into. From Table 6-27, it can be noted that the Petrifilm™ test yields a large percentage of FN's in the Conformity/Low and Intermediate Risk Levels (24% and 42%). Also, 6% of the samples that were classified as being under the "High/Very High" Risk Level were found to be negative by the Petrifilm™ test.

Table 6-27. Percentage of False Negatives per WHO Risk Level for the Petrifilm™ Test for Capiz and Cambridge Samples.

WHO Risk Level (CFU/100mL)	False Negative Results for the Petrifilm™ test (%)
Conformity/Low (<10) (n=104)	*24*
Intermediate (10-100) (n=48)	*42*
High/Very High (>100) (n=66)	*6*

6.7.1.3. Error and Proportional Reduction in Error, λ

The tables used in calculating the error and proportional reduction in error, λ, for improved and unimproved water sources are presented in Appendix I. 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. The actual error and λ values are presented in Table 6-31.

Table 6-28. Error and Proportional Reduction in Error for Petrifilm™ for Capiz samples.

Test	Unimproved Sources			Improved Sources		
	Error	λ	n[1]	Error	λ	n[2]
Petrifilm™	17.1%	-133%	41	13.5%	74.2%	126

[1]: Sample size for unimproved sources.
[2]: Sample size for improved sources.

For unimproved sources, the addition of Petrifilm™ did not reduce our error, but in fact increased it (λ = -133%). 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 our error by 74.2%, with an error of 13.5%. Therefore, as a single test for improved sources, the PetrifilmTM test is a useful, additional predictor of contamination.

The numbers presented in Table 6-28 are not identical to those presented in Section 5.7.1.1, since the sample size is different (Capiz samples only), and since the test results have been divided between unimproved and improved sources.

6.7.1.4. 2x2 Contingency Table with WHO Risk Levels

The 2x2 contingency table with WHO Risk Levels for the Petrifilm™ test compared to Quanti-Tray® test results is presented below in Table 6-29 for Capiz and Cambridge samples combined. This table presents the Petrifilm™ and Quanti-Tray® test results broken down into two categories: the WHO Risk Levels (Conformity to Intermediate, High/Very High).

Table 6-29. 2x2 Contingency Table for the Petrifilm™ Test Compared to Quanti-Tray® for Capiz and Cambridge Samples.

		Quanti-Tray®	
		Low to Intermediate	*High/Very High*
Petrifilm™	*Low to Intermediate*[1]	121	9
	High/Very High[1]	30	58

[1]: The WHO Risk Levels were determined based on the sample volume used in the Petrifilm™ test (1 mL) compared to the actual risk levels based on a 100 mL sample. Low to Intermediate: 0 CFU mL, High/Very High: >1 mL.

The majority of samples (179) were identically classified by the Petrifilm™ test and Quanti-Tray®. The true results percentage (i.e. results that lie in the same WHO Risk Level for the Petrifilm™ test and Quanti-Tray®) for this contingency table is 82%. However, what is most important here is that the WHO Risk Level for a given sample, obtained by the Petrifilm™ test, corresponds to the same or a lower-risk WHO Risk Level (shaded region in Table 6-29). In this light, the true results percentage (i.e. results that lie in the same or higher WHO Risk Level for the Petrifilm™ test than Quanti-Tray®) is 95%. It is important to mention that the Petrifilm™ test has a very high detection limit: a count of "0" on a Petrifilm™ could mean that the sample is free of *E.coli* (Conformity Risk Level) or that the water is unsafe to drink (Intermediate Risk Level). It is the lack of categorization at low (<100 CFU/100 mL) *E.coli* concentrations that are responsible for the high correlation value obtained for Petrifilm™ and Quanti-Tray®.

6.7.1.5. *Chi-Square and Fisher's Exact Tests*

Table 6-30 lists the values obtained from the Chi-square (χ^2 and p) and Fisher's exact test for the 2x2 contingency table with WHO Risk Levels for Petrifilm™ compared to Quanti-Tray® for Capiz and Cambridge samples. These statistical results were calculated using Stata® Release II software.

Table 6-30. Chi-Square Value, *p*-value, Fisher's Exact Test Probability and Statistical Significance for the 2x2 Contingency Table with WHO Risk Level for the Petrifilm™ Test Compared to Quanti-Tray® for Capiz and Cambridge Samples.

	Chi-square value	*p*-value	Fisher's exact test probability	Statistical significance
Petrifilm™ (n=218)	85.7687	0.000	0.000	Very highly significant

These results show that there is a very high significant statistical relationship between the Petrifilm™ test and Quanti-Tray®.

6.7.1.6. *Scatter Plot*

Figure 6-3 presents the scatter plot of Petrifilm™ test results against the Quanti-Tray® test results for Capiz and Cambridge test results combined (with Quanti-Tray®, detection limit = 200.5 MPN/100 mL). Figure 5-6 presents the scatter plot for Cambridge test results only (with Quanti-Tray®/2000 detection limit = 2419 MPN/100 mL). These graphs show a positive correlation between the Petrifilm™ and Quanti-Tray® test results.

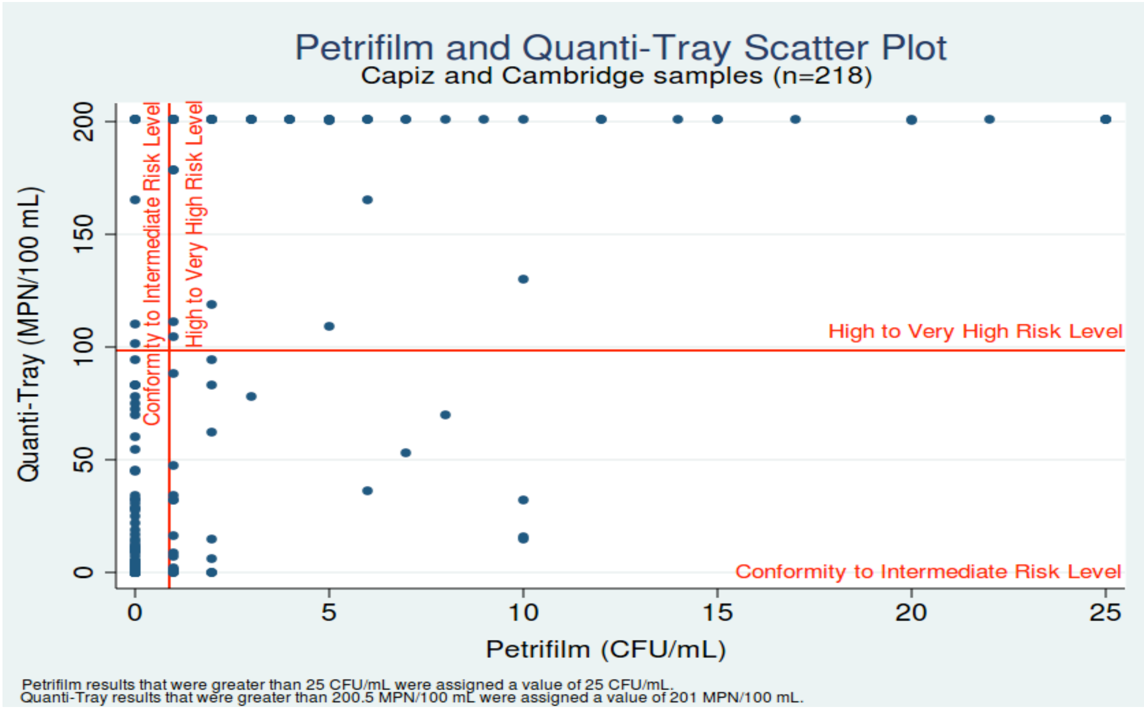

Figure 6-4. Petrifilm™ vs. Quanti-Tray® Scatter Plot with WHO Risk Levels for Capiz and Cambridge Samples.

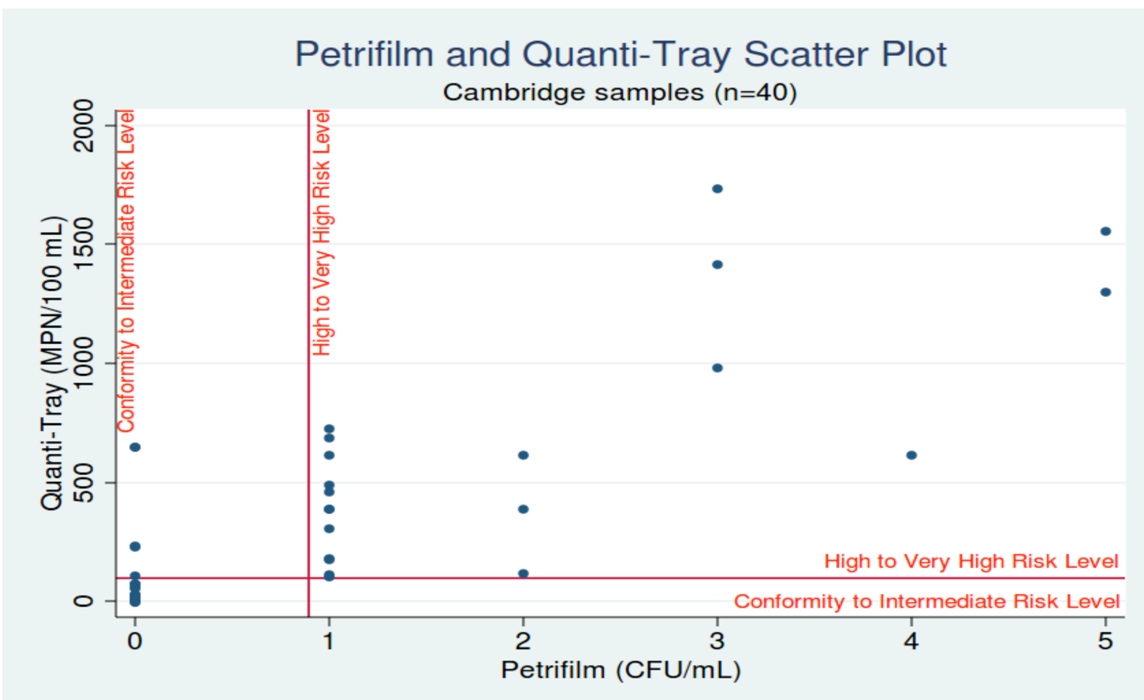

Figure 6-5. Petrifilm™ vs. Quanti-Tray® Scatter Plot with WHO Risk Levels for Cambridge Samples.

6.7.2. Compared to Membrane Filtration

6.7.2.1. 2x2 Contingency Table

The 2x2 contingency table for the Petrifilm™ test compared to membrane filtration test results is presented in Appendix H for Cambridge samples only. The corresponding TR, FP, FN, Sensitivity, Specificity, PPV and NPV values are presented in Table 6-31 below.

Table 6-31. Percentage of True and False Results, and Sensitivity, Specificity, PPV and NPV Results for Petrifilm™ Test Compared to Membrane Filtration for Cambridge Samples.

	True Results	False Positives	False Negatives	Sensitivity	Specificity	PPV	NPV
Petrifilm ™ (n=40)	58	0	43	55	100	100	11

In general, the same trend is noted here as in the comparison with Quanti-Tray® explained above. The Petrifilm™ test has a low percentage of TR (58%), 0% FP's and a high proportion of FN's (43%). The Specificity and PPV values are high (100%), whereas the Sensitivity and NPV values were low (55% and 11%, respectively). This also confirms the fact that the Petrifilm™ test is not a good indicator of the presence of *E.coli* contamination, but is a good indicator of the absence of *E.coli* contamination. Also, a positive result with the Petrifilm™ test is usually indicative of *E.coli* contamination, whereas a negative result is usually (89% of the time) not synonymous with absence of *E.coli* contamination.

The 100% results for the specificity and PPV criteria is probably due to high level of contamination present in Charles River (even in a 1 in 100 dilution), whereas samples collected in Capiz did not all have this high level of contamination.

6.7.2.2. Chi-Square and Fisher's Exact Tests

Table 6-32 lists the values obtained from the Chi-square (χ^2 and p) and Fisher's exact test for the 2x2 contingency table with WHO Risk Levels for Petrifilm™ compared to membrane filtration for Cambridge samples only. These statistical results were calculated using Stata® Release II software.

Table 6-32. Chi-Square Value, *p*-value, Fisher's Exact Test Probability and Statistical Significance for the 2x2 Contingency Table with WHO Risk Level for the Petrifilm™ Test Compared to Membrane Filtration for Cambridge Samples.

	Chi-square value	*p*-value	Fisher's exact test probability	Statistical significance
Petrifilm™ (n=40)	2.3269	0.127	0.219	Not statistically significant

These results show that there is not a statistically significant relationship between the Petrifilm™ test and membrane filtration. This is probably due to the small sample size (n = 40 samples) used in this correlation analysis.

6.8. EC-Kit

6.8.1. Compared to Quanti-Tray®

6.8.1.1. 3x3 Contingency Table

Table 6-33, which shows the combinations of Colilert and Petrifilm™ (EC-Kit) *E.coli* test results, and the associated WHO Risk Levels, was used to construct the 3x3 contingency table (Table 6-34), where EC-Kit test results were compared to Quanti-Tray® test results for Capiz and Cambridge samples. The results were broken down into three categories: the WHO Risk Levels (Conformity/Low, Intermediate and High/Very High).

Table 6-33. WHO Risk Levels and Corresponding EC-Kit Test Results (Adapted from WHO (1997) replacing "thermotolerant bacteria" with "*E.coli*").

WHO Risk Level	*E.coli* in sample (CFU/100 mL)	Colilert *E.coli* Result	Petrifilm™ *E.coli* 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 6-34. 3x3 Contingency Table for the EC-Kit Compared to Quanti-Tray® for Capiz and Cambridge Samples.

		Quanti-Tray®		
		Low/Conformity	*Intermediate*	*High/Very High*
EC-Kit	*Low/Conformity*	67	4	0
	Intermediate	20	16	4
	High/Very High	4	16	36

The majority of samples (119) were identically classified by the EC-Kit and Quanti-Tray®. The true results percentage (i.e. results that lie in the same WHO Risk Level for the EC-Kit and Quanti-Tray®) for this 3x3 contingency table is 55%. However, what is important here is that the WHO Risk Level for a given sample, obtained by the EC-Kit, corresponds to the same or a lower-risk WHO Risk Level (shaded region in Table 6-34). In this light, the true results percentage (i.e. results that lie in the same or higher WHO Risk Level for the EC-Kit test than Quanti-Tray®) is 96%. Again, such misclassifications err on the side caution as it can result in the rejection of water that may be safe to drink. This option is much better than misclassifying water that is not safe to drink as water that is.

6.8.1.2. Chi-Square and Fisher's Exact Tests

Table 6-35 lists the values obtained from the Chi-square (χ^2 and p) and Fisher's exact test for the 2x2 contingency table with WHO Risk Levels for EC-Kit compared to Quanti-Tray® for Capiz and Cambridge samples. These statistical results were calculated using Stata® Release II software.

Table 6-35. Chi-square Value, *p*-value, Fisher's Exact Test Probability and Statistical Significance for the 3x3 Contingency Table with WHO Risk Level for the EC-Kit Test Compared to Quanti-Tray® for Capiz and Cambridge Samples.

	Chi-square value	***p*-value**	**Fisher's exact test probability**	**Statistical significance**
EC-Kit (n=218)	n/a	n/a	0.000	Very highly significant

n/a: not applicable

Some cells are marked "n/a" because some cells in the contingency table contained values less than 5, therefore the chi-square test was not applicable. Instead, only Fisher's exact test was used to determine statistical significance. The result obtained from Fisher's exact test shows that there is a very significant statistical relationship between the EC-Kit and Quanti-Tray®.

6.8.2. Compared to Membrane Filtration

6.8.2.1. 3x3 Contingency Table

The 3x3 contingency table for the EC-Kit compared to membrane filtration is presented below in Table 6-36 for Cambridge samples only. This table presents the EC-Kit and membrane test results broken down into three categories: the WHO Risk Levels (Conformity/Low, Intermediate and High/Very High).

Table 6-36. 3x3 Contingency Table for the EC-Kit Compared to Membrane Filtration for Cambridge Samples.

		Membrane filtration		
		Conformity/Low	*Intermediate*	*High/Very High*
EC-Kit	*Conformity/Low*	2	0	0
	Intermediate	5	8	4
	High/Very High	0	3	18

The majority of samples (28) were identically classified by the EC-Kit and membrane filtration. The true results percentage (i.e. results that lie in the same WHO Risk Level for the EC-Kit and membrane filtration) for this 3x3 contingency table is 70%. However, what is important here is that the WHO Risk Level for a given sample, obtained by the EC-Kit, corresponds to the same or a lower-risk WHO Risk Level (shaded region in Table 6-36). In this light, the true results percentage (i.e. results that lie in the same or higher WHO Risk Level for the EC-Kit test than membrane filtration) is 90%. Again, such misclassifications err on the side caution as it can result in the rejection of water that may be safe to drink. This option is much better than misclassifying water that is not safe to drink as water that is.

6.8.2.2. Chi-Square and Fisher's Exact Tests

Table 6-37 lists the values obtained from the Chi-square (χ^2 and p) and Fisher's exact test for the 2x2 contingency table with WHO Risk Levels for EC-Kit compared to membrane filtration for Cambridge samples only. These statistical results were calculated using Stata® Release II software.

Some cells are marked "n/a" because some cells in the contingency table contained values less than 5, therefore the chi-square test was not applicable. Instead, only Fisher's exact test was used to determine statistical significance. The result obtained from Fisher's exact test shows that there is a very significant statistical relationship between the EC-Kit and membrane filtration.

Table 6-37. Chi-Square Value, *p*-value, Fisher's Exact Test Probability and Statistical Significance for the 3x3 Contingency Table with WHO Risk Level for the EC-Kit Test Compared to Membrane Filtration for Cambridge Samples Only.

	Chi-square value	*p*-value	Fisher's exact test probability	Statistical significance
Petrifilm (n=218)	n/a	n/a	0.000	Very highly significant

n/a: not applicable

6.9. Test combinations

Since the EC-Kit gave much better results than the Petrifilm™ and Colilert tests alone, the accuracy of different combinations of P/A test and enumerative test was analyzed. These combinations were compared statistically to Quanti-Tray® using the 3x3 contingency table and again looking at the percentage errors, and proportional reduction in error. The test combinations are presented in Table 6-38.

Table 6-38. P/A and enumerative test combinations.

Test Combinations
Colilert + Petrifilm™ (EC-Kit)
10-mL H_2S test + Petrifilm™
20-mL H_2S test + Petrifilm™
100-mL H_2S test + Petrifilm™
20-mL HACH test + Petrifilm™
Colilert + Easygel®
10-mL H_2S test + Easygel®
20-mL H_2S test + Easygel®
100-mL H_2S test + Easygel®
20-mL HACH test + Easygel®

6.9.1. New Risk Levels

Like Table 6-33, which depict EC-Kit (Colilert + Petrifilm™) test results and corresponding WHO Risk Levels, similar tables were set up for the different test combinations and are presented here for H_2S test + Petrifilm™ (Table 6-39), Colilert + Easygel® (Table 6-40) and H_2S test + Easygel® (Table 6-41). The corresponding WHO Risk Levels for Easygel® were for a sample volume of 5 mL.

Table 6-39. WHO Risk Levels and Corresponding H_2S Test + Petrifilm™ Results (Adapted from WHO (1997) replacing "thermotolerant bacteria" with "*E.coli*").

WHO Risk Level	H_2S Test Result	Petrifilm™ Result (CFU/mL)
Conformity	Yellow	0
Low	Yellow	0
Intermediate	Black	0
High	Black	1-10
Very High	Black	>10

Table 6-40. WHO Risk Levels and Corresponding Colilert + Easygel® Test Results (Adapted from WHO (1997) replacing "thermotolerant bacteria" with "*E.coli*").

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 6-41. WHO Risk Levels and Corresponding H_2S Test + Easygel® Results (Adapted from WHO (1997) replacing "thermotolerant bacteria" with "*E.coli*").

WHO Risk Level	H_2S Test Result	Easygel® (CFU/5 mL)
Conformity	Yellow	0
Low	Yellow	0
Intermediate	Black	0-4
High	Black	5-50
Very High	Black	>50

It should be noted that the new 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.

6.9.2. Error and Proportional Reduction in Error

The 3x3 contingency table for the test combinations for improved and unimproved sources, compared to Quanti-Tray®, are presented in Appendix I. 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. The corresponding error and proportional reduction in error, λ, are presented in Table 6-42. 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 6-42. Error, Proportional Reduction in Error, λ, and sample number, n, for unimproved and improved sources, for samples collected in Capiz and compared to Quanti-Tray®.

	Unimproved Sources			Improved Sources		
	Error	**λ**	**n[1]**	**Error**	**λ**	**n[2]**
Colilert (EC-Kit) + Petrifilm™	3.6%	51%	28	4.8%	90%	126
10-mL H_2S test + Petrifilm™	9.1%	82%	33	3.5%	93%	114
20-mL H_2S test + Petrifilm™	12.1%	-33%	33	2.4%	95%	126
100-mL H_2S test + Petrifilm™	6.1%	33%	33	1.6%	97%	125
20-mL HACH test + Petrifilm™	15.2%	-67%	33	1.6%	97%	125
Colilert + Easygel®	0.0%	100%	13	0.0%	100%	28
10-mL H_2S test + Easygel®	0.0%	100%	4	0.0%	100%	18
20-mL H_2S test + Easygel®	0.0%	100%	4	0.0%	100%	19
100-mL H_2S 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.

In general, for unimproved and improved sources, the combination of tests yielded better prediction of fecal contamination than single tests, with the exception of 20-mL H_2S test + Petrifilm™ (λ = -33% for unimproved sources) and 20-mL HACH test + Petrifilm™ (λ = -67% for unimproved sources), in other words, the 20-mL H_2S test or the assumption of contamination based on source type, are better predictors than the 20-mL H_2S test + Petrifilm™ and 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 Easygel® was combined with. As a matter of fact, the Easygel® test yielded little FP results (1%) and a many FN results (17%). On the other hand, the H_2S 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).

6.10. Summary of Statistical Analyses

As previously mentioned, the primary objective of this study was to assess the accuracy of the H_2S tests (laboratory-made: 10-, 20- and 100-mL sample volume and industry-made HACH 20-mL sample volume), Easygel®, Colilert and Petrifilm™ (EC-Kit), through comparison with two standard method tests: the Quanti-Tray® and membrane filtration; and to provide recommendations on the suitability of H_2S-producing bacteria as a valid indicator of fecal contamination.

The following briefly summarizes the statistical analyses' key findings for the field-based, microbiological tests as single tests, and as test combinations.

6.10.1. H_2S Test

Through correlation analyses, it was shown that the H_2S test results (for laboratory-made reagent: 10-, 20- and 100-mL sample volume and industry-made HACH test 20-mL sample volume) were correlated, in a statistically significant way with Quanti-Tray®. Statistical correlation with another Standard Methods Test (membrane filtration) was not proven in this study because of the small sample size used (n = 40 samples), although a trend toward statistical significance was noted.

In general, all the H_2S tests had high true results values, although the 20-mL laboratory-made H_2S test had the highest percentage of true results (84%) when tests were compared to Quanti-Tray®. The FP values for the H_2S tests were high (9% to 16% for the 10- and 100-mL sample volume, respectively); whereas the FN values for the H_2S tests were low (4 to 11% for the 100- and 10-mL sample volume, respectively). The high percentage of FP results is probably due to the H_2S tests detecting H_2S that may not come from H_2S-producing fecal bacteria. For example, in groundwater, H_2S is often present due to natural geohydrological sources and to anthropogenic impacts other than fecal contamination (Sobsey & Pfaender, 2002). This phenomenon is particularly of interest in this study since most drinking water samples from Capiz Province (136 samples) were groundwater collected from wells and spring sources.

It was noted that as the sample volume of the H_2S test increased, sensitivity also increased from 84% for the 10-mL test to 94% for the 100-mL test, which means that the higher volume test can detect more true positives; whereas specificity decreased considerably from 72% for the 10-mL test to 53% for the 100-mL test; which means that the higher volume test detects less true negative results.

Also, the PPV value for the 10- and 20-mL tests were similar at 85% to 86%, but was much smaller for the 100-mL test (53%); in other words, when a larger sample volume is used, a positive test is no longer directly synonymous with presence of fecal contamination. Finally, NPV increased with increasing sample volume from 69% for the 10-mL test to 82% for the 100-mL test, which means that a when a larger sample volume is used, a negative test becomes more likely to reflect true absence of fecal contamination.

The detection limit of the H_2S tests was also evaluated. As expected, it was found that the 100-mL H_2S test had the lowest detection limit (7.5 MPN/100 mL), whereas the other H_2S tests (with smaller sample volumes) all failed to detect samples that had an *E.coli* concentration greater than 45 MPN/100 mL (i.e. Intermediate Risk Level).

Finally, the error and proportional reduction in error were calculated based on the following initial assumptions:

- The U.N.-designated unimproved water sources were all contaminated (High/Very High Risk Level or Presence of contaminant)
- The U.N.-designated improved water sources were all safe (Conformity/Low Risk Level or Absence of contaminant)

It was found that, for unimproved sources, the laboratory-made H_2S tests had a 9% error and 0% proportional reduction in error. This means that **for unimproved sources, the addition of the laboratory-made H_2S tests did not improve the error**. The addition of the HACH H_2S test had a 21.2% error and a -133% proportional reduction in error, which means that the HACH H_2S test actually **increased the error**. However, for improved sources, the H_2S tests (laboratory-made and HACH) had an error that ranged from 20% to 29% and a 61% to 44% reduction in error for the 20-mL and 100-mL laboratory-made H_2S test, respectively. This means that **for improved sources, the addition of the H_2S tests (laboratory- and industry-made) improved the error**.

6.10.2. Easygel®

Through correlation analyses, it was shown that Easygel® test results were correlated, in a statistically significant way with both Quanti-Tray® and membrane filtration.

The Easygel® test had a high TR value (81%), few FP's (1%) and many FN's (17%), when compared to Quanti-Tray®. The Sensitivity and NPV values for Easygel® were relatively low (78% and 55%, respectively), which means that the Easygel® test is not a particularly good indicator of the presence of contamination, and a negative Easygel® test result is at times (45% of the time) synonymous with absence of *E.coli*. However, the Easygel® test yields high Specificity and PPV values, which means that it is a particularly good indicator of the absence of contamination, and that a positive test result is usually indicative of the *E.coli* presence.

The detection limit of the Easygel® was also evaluated. It was found that the Easygel® test had a high detection limit since it failed to detect the presence of *E.coli* in samples that were in the Intermediate Risk Level (*E.coli* concentration greater than 10 MPN/100 mL).

Finally, the error and proportional reduction in error were calculated. It was found that, for unimproved sources, Easygel® had a 29% error and -100% proportional reduction in error.

This means that the **Easygel® test yields a less accurate prediction than predicting that all unimproved sources are contaminated**. However, for improved sources, the Easygel® test had a 25% error and a 52% reduction in error. This means that **for improved sources, the Easygel® test improved the error.**

6.10.3. Colilert

Through correlation analyses, it was shown that Colilert test results were correlated, in a statistically significant way with both Quanti-Tray® and membrane filtration.

The Colilert test had a high TR value (83%), few FP's (5%) and a somewhat low proportion of FN's (11%), when compared to Quanti-Tray®. The Sensitivity, Specificity and PPV values were all relatively high (83%, 84% and 92%, respectively), which means that the Colilert test is a particularly good indicator of the presence of contamination. However, the Colilert test had a lower NPV value (70%), which means that the Colilert test at times (30% of the time) yields a negative result although there is presence of *E.coli*.

The detection limit of the Colilert was also evaluated. It was found that the Colilert test had a high detection limit since it failed to detect the presence of *E.coli* in many samples that had an *E.coli* concentration of 62.4 MPN/100 mL (Intermediate Risk Level). It must also be noted that the Colilert test failed to detect the presence of *E.coli* in a water sample that was in the High/Very High Risk Level (*E.coli* concentration greater than 100 MPN/100 mL).

Finally, the error and proportional reduction in error were calculated. It was found that, for unimproved sources, Colilert had a 5% error and 33% proportional reduction in error. However, for improved sources, the Colilert test had a 22% error and a 58% reduction in error. This means that the Colilert test is an **accurate test to determine the presence or absence of *E.coli* in unimproved and improved sources**, and greatly improves the initial predictions based on water source level alone.

6.10.4. Petrifilm™

Through correlation analyses, it was shown that the Petrifilm™ test results were correlated, in a statistically significant way with Quanti-Tray® and were not correlated in a statistically significant way with membrane filtration. The latter is probably due to the small sample size (n = 40 samples) used in this correlation analysis.

The Petrifilm™ test had a low TR value (67%), few FP's (3%) and many FN's (30%), when compared to Quanti-Tray®. The Specificity and PPV values were high (91% and 93%, respectively), whereas the Sensitivity and NPV values were low (55% and 49%, respectively). This means that Petrifilm, like the Easygel® test, is a good indicator of the absence of *E.coli* contamination, but not a good indicator of the presence of *E.coli* contamination. Also, a positive result with the Petrifilm™ test is usually indicative of *E.coli* contamination, whereas a negative result is typically (51% of the time) not synonymous with absence of *E.coli* contamination.

The detection limit of the Petrifilm™ was also evaluated. It was found that the Petrifilm™ test had a very high detection limit since it failed to detect the presence of *E.coli* in many samples were in the Intermediate Risk Level (*E.coli* concentration greater than 10 MPN/100 mL) and High/Very High Risk Level (*E.coli* concentration greater than 100 MPN/100 mL). In fact, 42% of samples that were tested and ranked in the Intermediate Risk Level, had negative Petrifilm™ test results.

Finally, the error and proportional reduction in error were calculated. It was found that, for unimproved sources, Petrifilm™ had a 17% error and a -133% proportional reduction in error. This means that **the Petrifilm™ test yields a less accurate prediction than predicting that all unimproved sources are contaminated**. However, for improved sources, the Petrifilm™ test had a 14% error and a 74% reduction in error. This means that **the Petrifilm™ test is an accurate test to determine the presence or absence of *E.coli* in improved sources only**.

6.10.5. Test Combinations

Through correlation analyses, it was shown that the EC-Kit test results were much more accurate than the individual Colilert and Petrifilm™ test results. As such, test results of different testing combinations of one P/A test with one enumerative test were also analyzed. The following test combinations were evaluated:

- Colilert + Petrifilm™ (EC-Kit)
- 10-mL H2S test + Petrifilm™
- 20-mL H2S test + Petrifilm™
- 100-mL H2S test + Petrifilm™

- 20-mL HACH test + Petrifilm™
- 10-mL H2S test + Petrifilm™
- 20-mL H2S test + Petrifilm™
- 100-mL H2S test + Petrifilm™
- 20-mL HACH test + Petrifilm™
- Colilert + Easygel®
- 10-mL H2S test + Easygel®
- 20-mL H2S test + Easygel®
- 100-mL H2S test + Easygel®
- 20-mL HACH test + Easygel®

Also, WHO Risk Levels corresponding to the different combined test outcomes of the testing combinations were established.

The testing combinations were evaluated based on the error and proportional reduction in error. In general, **it was shown that for both improved and unimproved water sources, most of the test combinations yielded more accurate results than single tests**. This would mean that the tests in a given test combination complemented one another. This is especially true of the H_2S test and Easygel® combination where the H_2S test had a high proportion of FP and a low proportion of FN results; whereas the Easygel® test had a high proportion of FN and a low proportion of FP results.

It is interesting to note that all test combinations that included Easygel® had 100% accurate test results (0% error). Although these are highly promising results, it is worth noting that the sample size for the Easygel® test combinations was particularly small (n = 3 to n = 28 samples).

7. Other Factors: Cost, Practicality/Ease of Use

As mentioned in the Chapter 1 of this thesis, the new field-based microbiological tests will also be assessed based on the following factors: cost and practicality/ease.

7.1. Total Cost

The cost summaries presented here are only for the new, field-based microbiological tests: H_2S test, Easygel® and EC-Kit (Colilert and 3M™ Petrifilm™). The cost of the Standard Methods tests (Quanti-Tray® and membrane filtration) were not included in this chapter 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, these tests are expensive (Quanti-Tray® tests can range from $6 to $21 per sample) and require the use of many, and at times expensive equipment (sealer, vacuum pump, glassware and filtration unit set-up for membrane filtration).

7.1.1. H_2S test

7.1.1.1. Laboratory-Made Reagents

Variable Cost

The H_2S test, or the H_2S paper strip test, requires the use of readily available laboratory reagents, distilled or de-ionized water, and paper towels or toilet paper.

The US$ price for the reagents is listed below in Table 7-1. 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 States. The total price listed represents the price of reagents required to make 2.5 L of H_2S reagent solution (5,000 tests for the 10-mL H_2S test, 2,500 for the 20-mL H_2S test and 1,000 for the 100-mL H_2S 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.

It is important to note that the price of reagents in the Philippines is almost 2.5 times more expensive than the price of the same reagents in the United States.

Table 7-1. Reagents Required for H_2S test, Amount Required for 100 mL of Reagent Solution and Price.

Reagents	Amount required[1]	Amount (/unit)	Price in US ($/unit)	Price in Philippines[2] ($/unit)
Bacteriological peptone	40.0 g	1,000 g	207.00	523.60
Dipotassium hydrogen phosphate	3.00 g	100 g	20.30	47.77
Ferric ammonium citrate	1.50 g	100 g	37.20	94.16
Sodium thiosulphate	2.00 g	500 g	21.78	17.62[3]
Sodium lauryl sulfate	0.20 g	25 g	29.90	70.53
L-cystine (for M2 medium only)	0.25 g	25 g	28.00	76.01
		TOTAL	**344.18**	**829.69**

[1]: The amount required comes from recipes provided by (Manja, Maurya, & Rao, 1982), (Venkobachar, Kumar, Talreja, Kumar, & Iyengar, 1994) and (Grant & Ziel, 1996). This includes the addition of L-cystine and replaces 1 mL of Teepol by 0.20 g of sodium lauryl sulfate.
[2]: Price of these reagents in Philippines was also obtained through the Sigma Aldrich and VWR websites.
[3]: This price was listed on the VWR website was £11.40 for 500g of sodium thiosulphate, which converts to US$17.62 as per the exchange rate on April 14, 2010.

Another important element of the H_2S test is the paper strip. These strips of paper must be non-toxic absorbing paper, which include paper towels and toilet paper. Here, we will solely be looking at the cost and surface area of paper towels. In the United States, a roll of paper towel costs approximately $1.50 whereas in the Philippines, a roll costs $0.80 (PHP40).

Fixed Cost

Furthermore, for the 10- and 20-mL H_2S tests, samples are usually tested in vials, which can be washed, sterilized and reused continuously. These vials/bottles must be made of clear glass (for sterilization in oven or autoclave and for easy interpretation of results), with a black polypropylene screw top. Table 7-2 lists the cost of vials for 10-mL and 20-mL vials available from Sigma Aldrich, and the cost of bottles for 100-mL bottles available from VWR.

Table 7-2. Cost of Bottles/Vials for H_2S 10-, 20- and 100-mL Tests.

Volume of vial/bottle	Bottles/vials per pack	Price in US/pack ($)	Price in US/vial or bottle ($)	Price in Philippines /pack ($)	Price in Philippines/vial or bottle ($)
10 mL	100	102.50	1.03	259.60	2.60
20 mL	100	116.00	1.16	293.71	2.94
100 mL	12	134.50	11.21	316.72	26.39

Alternately, disposable, sterile sampling bags with a wire top and white marking area can be used for the 100-mL H_2S test. These are available from VWR for $44.54 for 1 pack of 500 bags in the United States, and $76.12[1] for 1 pack of 500 bags in the Philippines.

Although the initial fixed cost of vials/bottles is high, if many (e.g. 2,500) tests are performed, the average cost of vials/test is significantly reduced (approximately 4¢ and 8¢ for the 10-mL test, 5¢ and 12¢ for the 20-mL test and 5¢ and 13¢ for the 100-mL test, in the United States and Philippines, respectively). On the other hand, 100-mL sterile sampling bags provide an interesting, and perhaps less expensive alternative if fewer tests (less than 2,500) are conducted (9¢ and 15¢ in the United States and Philippines, respectively).

The HACH P/A media is available directly from the HACH website. Typically, one pouch (called "powder pillow") is used as the test reagent for a 20-mL sample volume. A pack of 50 powder pillows is $29.39, or approximately 59¢ per test. No data was available on the HACH website as to the price of the HACH P/A PathoScreen in developing countries or in the Philippines.

The HACH sample was tested in clear glass vials, with a black polypropylene screw top, identical to the ones used in the H_2S 20-mL sample test.

Average Cost per Test

The following Table 7-3 presents the average cost per test for the 3 different laboratory-made H_2S tests and HACH test, from a 2.5 L reagent solution (cost calculated in Table 7-1) for just the test reagent themselves, not including the sample vial, bottle or sampling bag).

Table 7-3. Average Cost per Test for Different H_2S Test Sample Volumes, from a 2.5 L Reagent Solution

	Laboratory-made H_2S Test Sample Volume			HACH test
	10 mL	**20 mL**	**100 mL**	**20-mL**
Reagent volume/test (mL)	0.5	1.0	2.5	n/a
No. of paper rolls/test[1]	1	1	1	n/a
No. of samples tested	5,000	2,500	1,000	n/a
United States - Average cost/test[2] ($)	**0.07**	**0.14**	**0.35**	**0.59**
Philippines - Average cost/test[2] ($)	**0.17**	**0.33**	**0.83**	**n/a**

n/a: not applicable
1: Number of paper rolls per tests from 2.5 L reagent solution is obtained by dividing the average area required to adequately absorb the reagent volume (2x3 cm2 for 0.5 mL sample, 4x3 cm2 for 1.0 mL sample and 2- 4x3 cm2 for 2.5 mL sample) divided by the average area per paper roll (52 ply of 11'' x 11 '' ≈ 40,500 cm2/roll).
2: The average cost per test was calculated based on the cost of laboratory reagents for 2.5 L of solution listed in Table 7-1, adding the cost of the paper towels. The cost of vials/bottles and sampling bags was not included in the average cost/test.

It is important to note that the price of reagents in the Philippines is almost 2.5 times more expensive than the price of the same reagents in the United States.

The least expensive H_2S test is the 10-mL test, which costs approximately 7¢/test to conduct in the United States, or 17¢/test to conduct in the Philippines. The 20-mL test is twice as expensive as the 10-mL test and costs 14¢/test and 33¢/test to conduct in the United States and in the Philippines, respectively. Finally, the 100-mL test is the most expensive test: 35¢/test and 83¢/test to conduct in the United States and in the Philippines, respectively, because it requires a larger reagent volume.

7.1.1.2. Other Considerations

Other elements to consider which were not included in the cost analysis of the H_2S paper strip test are the use of distilled water: how much does water cost in areas around the world (piped water, bottled water)? Or how far does one have to walk to fetch water? Or the costs associated with boiling the water.

Finally, the figures cited here represent an approximate cost of each H_2S test. It is important to consider that prices differ greatly from country to country, and that cost of reagents and laboratory supplies are usually more expensive in developing countries. Also, it is important to consider freight/transportation costs associated with shipping the reagents to remote locations worldwide.

7.1.2. 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 10 tests for $21.25/set if 1 to 9 sets are purchased and for $16.25/set if more than 10 sets are purchased. This means that individual tests range from $1.63 to $2.13. No data was available on the Micrology Laboratories website as to the price of Easygel® in developing countries or in the Philippines.

7.1.3. 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 Whirl-Pak bags, individually wrapped, sterile pipettes, a UV lamp with 4 AA batteries, an insulated cooler bag, and laminated instructions. The additional contents and the price of each kit model are described in Table 7-4. These costs do not include cost of domestic U.S. postage, which can range from $5 to $20 depending on the kit size and speed of delivery; or even the cost of international postage.

Table 7-4. Contents and Cost of EC-Kit Model A, B, C and D.

	Kit contents	Total cost ($)	Number of tests	Cost/test ($)
Model A (C-10)	▪ 10 Colilert tests	32.00	10	3.20
Model B (CP-25)	▪ 25 Colilert tests ▪ 25 3M™ Petrifilm™ (1 pack) ▪ Incubator belt ▪ 2 ice packs ▪ 10 cardboard squares ▪ 20 rubber bands	104.00	25	4.16
Model C (CP-50)	▪ 50 Colilert tests ▪ 50 3M™ Petrifilm™ (1 pack) ▪ Incubator belt ▪ 2 ice packs ▪ 20 cardboard squares ▪ 40 rubber bands	187.00	50	3.74
Model D (CP-100)	▪ 100 Colilert tests ▪ 100 3M™ Petrifilm™ (1 pack) ▪ Incubator belt ▪ 2 ice packs ▪ 30 cardboard squares ▪ 60 rubber bands	349.00	100	3.49

7.1.4. Cost Comparison

The following Table 7-5 compares the cost of each microbiological test. The H_2S tests (10-, 20-, 100-mL and HACH) were by far the least expensive of the microbiological tests presented here (less than 60¢ each), excluding the initial cost of glass vials and bottles or 100-mL sterile sampling bags. The Easygel® tests however, do not require the use of additional vials or bottles as a Petri dish is provided for each test.

Although EC-Kit has the highest cost per test, it should be noted that EC-Kit provides both P/A and enumerative data for two tests and related supplies, whereas the H_2S test and Easygel® provide solely P/A or enumerative information, respectively.

Table 7-5. Cost/test of H_2S test, Easygel® and EC-Kit.

Test	H_2S test				Easygel®		EC-Kit			
	10 mL[1]	20 mL[1]	100 mL[1]	HACH	1-9 sets	10+ sets	Model A	Model B	Model C	Model D
Cost/test ($)	0.07	0.14	0.35	0.59	2.13	1.63	3.20	4.16	3.74	3.49

[1]: The cost data presented in this table for the H_2S test reflects cost incurred in the United States in order to provide an adequate comparison with the Easygel® and EC-kit, since the costs of these tests if purchased in the Philippines was unavailable.

7.2. Practicality/Ease of use

The practicality/ease of use of the microbiological tests were rated based on the following 7 criteria:

1. Ease of training for test users: testers and readers
2. Ease of acquiring/making reagents
3. Ease of transportation, storage, and disposal of samples and tests
4. Ease of processing samples
5. Short incubation times
6. Use of electric incubator
7. Easy-to-read results

Each microbiological test was rated based on the above-listed criteria and was given a numerical score from 1 (Very Poor) to 5 (Very Good), which depended on how much each test satisfied the specific criteria.

7.2.1. H_2S Test

7.2.1.1. Laboratory-Made Reagents

- Ease of training for test users: testers and readers

The H_2S test is simple to use. Testers must simply place the H_2S test strip into the 10-, 20- or 100-mL vial/bottle, or 100-mL sterile sampling bag, pour the water sample in, and close and shake the sample. Readers must simply record the change of color from yellow to black. If the sample is black, the reader must record the sample as positive and if the sample remains yellow, the reader must record the sample as negative.

Score = 5

- Ease of acquiring/making reagents

The H_2S test is relatively easy to make: the ingredients can be found in any laboratory supply store, and the recipe is straightforward. In fact, a great benefit of the laboratory-made H_2S test reagent can be made in-country, by someone with basic lab skills, with access to a kitchen or laboratory. However, the laboratory-made H_2S test reagent requires time to make: vials need to be sterilized in the oven or autoclave first, then the liquid reagent needs to be prepared before it can be pipetted onto a paper strip, placed into the sterilized vial/bottle, and then heated in the oven for 1 hour. Therefore the average time to prepare the H_2S test strip reagent depends on the sample volume to be analyzed (or vial/bottle volume), and the size of the oven or autoclave. The H_2S test also requires access to a laboratory oven or autoclave, or at minimum a kitchen oven and thermometer.

Score = 2

- Ease of storage, transportation and disposal of samples and tests

One of the main benefits of the H_2S test is that it can be stored in a cool dry place for a maximum of 6 months (IDRC, 1998). The H_2S test strips are usually transported in their respective vials/bottles. This makes it harder to transport and travel with since it takes up more space, and there is always the possibility that vials/bottles may break. For example, the test vials/bottles can be placed in an autoclave for 15 minutes at 15 to 20 lbs of pressure; or the test samples can be sprayed with a disinfectant (e.g. household bleach), sit for 20 to 30 minutes before it can poured down the drain. If reusable glass vials/bottles are being used, these must be washed carefully with soap and water. Note that before these vials can be re-used, they must first be sterilized in an oven, autoclave or in boiling water.

Score = 3

- Ease of processing samples

H_2S test samples are easy to process: simply pour the water sample into the vial/bottle that contains the H_2S paper strip and incubate for 24 hours.

Score = 5

- Incubation times

The addition of L-cystine to the original M1 medium has significantly shortened the incubation times of the H_2S test. Although protocol dictates that samples should be verified after 24 hours of incubation, (Kromoredjo & Fujioka, 1991) noted that test results are seen rapidly, often after 12 to 15 hours of incubation.

Score = 5

- Use of electric incubator

(Pillai, Mathew, Gibbs, & Ho, 1999) showed that the H_2S bacteria test was most effective when carried out at temperatures between 22°C and 44°C. This means that, in tropical countries, the test can be performed at room temperature and does not require the use of an electric incubator or body heat incubation.

Score = 5

- Easy-to-read results

The H_2S test results simply record the change of color from yellow to black. If the sample is black, then the sample is positive for H_2S-producing bacteria and if the sample remains yellow, then the sample is negative for H_2S-producing bacteria.

Score = 5

7.2.1.2. HACH PathoScreen™ test

- Ease of training for test users: testers and readers

The HACH PathoScreen test is simple to use. Testers must aseptically open the HACH Powder Pillow and pour the contents into the 20-mL vial, which contains the water sample. Readers must record the change of color from yellow to black. If the sample is black, the reader must record the sample as positive and if the sample remains yellow, the reader must record the sample as negative.

Score = 5

- Ease of acquiring/making reagents

The HACH PathoScreen test can be purchased online from the HACH website or from its worldwide distributors.

Score = 5

- Ease of storage, transportation and disposal of samples and tests

The HACH PathoScreen powder pillows can be stored for approximately 1 year (the expiration date is indicated on the Certificate of Analysis that comes with each pack, and on each powder pillow). The fact that the HACH PathoScreen is a dehydrated medium, sterilized and individually packaged makes it particularly easy to transport, and unlike the H_2S test, it does not have to be transported in its respective vial/bottle. The disposal protocol for the HACH medium is identical to the H_2S test disposal protocol: samples must be sterilized before they can be disposed of. For example, the test vials/bottles can be placed in an autoclave for 15 minutes at 15 to 20 lbs of pressure; or the test samples can be sprayed with a disinfectant (e.g. household bleach), sit for 20 to 30 minutes before it can poured down the drain. If reusable glass vials/bottles are being used, these must be washed carefully with soap and water. Note that before these vials can be re-used, they must first be sterilized in an oven, autoclave or in boiling water.

Score = 4

- Ease of processing samples

The HACH PathoScreen™ P/A test is easy to process: simply aseptically open the HACH Powder Pillow and pour the contents into the 20-mL vial, which contains the water sample and incubate for 24 hours.

Score = 5

- Incubation times

The HACH PathoScreenTM sample must be incubated at a constant temperature for 24 to 48 hours (HACH, 2000). The incubation time for this medium is significantly longer than the incubation time for the H2S test.

Score = 3

- Use of electric incubator

In the HACH PathoScreenTM testing procedure, (HACH, 2000) showed that the test sample must be incubated at constant temperature between 25°C and 34°C. This means that, in tropical countries, the test can be performed at room temperature and does not require the use of an electric incubator

Score = 5

- Easy-to-read results

Similar to the H_2S test, the HACH PathoScreen™ test results simply record the change of color from yellow to black. If the sample is black, then the sample is positive for H_2S-producing bacteria and if the sample remains yellow, then the sample is negative for H_2S-producing bacteria.

Score = 5

7.2.2. Easygel®

- Ease of training for test users: testers and readers

Even though the Easygel® test is user-friendly, it is slightly more complicated than the H2S or HACH PathoScreenTM test. Testers must first pour 0.5 to 5 mL of sample into the

Easygel® media bottle, swirl the bottle and then pour into the pre-treated Petri dish. The liquid must first gel (about 20 minutes) before it can be incubated. Readers must count and record the number of blue (*E.coli*) and red (total coliform) colonies as CFU/mL of sample tested. This can be especially difficult if many colonies are present and/or overlap. Training for Easygel® is estimated to at approximately 30 minutes.

Score = 4

- Ease of acquiring/making reagents

The Easygel® tests can only be purchased from Micrology Laboratories, a company based in Indiana. Although their products can be purchased via the web, phone or by fax, ordering from and shipping to a remote area in a developing country might be difficult.

Score = 3

- Ease of storage, transportation and disposal of samples and tests

Ideally, the Easygel® media bottles must be kept frozen until time of use, although (Micrology Laboratories, 2009) state that media bottles can be thawed up to a month before use. This makes storage and transportation difficult, especially if tests are to be conducted for longer than a one-month period where reliable electricity is not always readily available. Easygel® samples and Petri dishes can by disposed of by bleaching the plates and throwing the sterilized gel media down the drain or in the garbage.

Score = 3

- Ease of processing samples

Easygel® tests are processed by pipetting 0.5 to 5-mL of the water sample into the Easygel® reagent bottle, where the mixture is swirled, and then poured into the pre-treated Petri dish. Once it has gelled, the Petri dish can be incubated.

Score = 4

- Incubation times

Depending on the incubation temperature, Easygel® test results can be counted after 24 to 48 hours of incubation.

Score = 4

- Use of electric incubator

One of the added benefits of the Easygel® test is that incubation temperature is not critical. The suggested temperature range is between 30°C and 37°C at which temperature the total coliform and *E.coli* colonies will grow faster than at incubation temperature from 22°C to 27°C. Results can be counted after 24 hours of incubation. This means that, in tropical countries, the test can be performed at room temperature and does not require the use of an electric incubator

Score = 5

- Easy-to-read results

The Easygel® test results record the number of blue (*E.coli*) and red (total coliform) colonies present in a certain volume of sample. Counting these colonies may be difficult, especially if many colonies are present and/or overlap.

Score = 4

7.2.3. EC-Kit

- Ease of training for test users: testers and readers

Even though the EC-Kit is meant to be user friendly, many testers and readers have found it difficult to use and interpret results. For the Colilert test, testers must first pour 10 mL of sample into the vial that already contains the Colilert reagent, invert the tube a few times to ensure media has completely dissolved, before it can be incubated. Readers must then record the change of color and fluorescence of the sample: clear (absence of total coliform) to yellow (presence of total coliform) and from non-fluorescence (absence of *E.coli*) to fluorescent (presence of *E.coli*) under UV light. For the Petrifilm™ test, testers must carefully pipette 1 mL of sample gently dispense it onto the center of the pink agar circle. The top film must then gently be rolled onto the Petrifilm™ plate, without trapping air bubbles under the top film. Once the water has naturally spread out to fill the entire pink circle and has been setting for 1 to 2 minutes, the film can be placed between two pieces of cardboard and incubated. Readers must count and record the number of blue (*E.coli*) and red (total coliform) colonies as CFU/mL. This can be especially difficult if many colonies are present and/or overlap. Basic training for the EC-Kit usually lasts from 15 to 30 minutes.

Score = 3

- Ease of acquiring/making reagents

Currently, the EC-Kit is only available through Susan Murcott at MIT, as part of a research and mapping project (of which this thesis is a part). Although it is possible to order EC-Kits online and by phone, ordering from and shipping to a remote area in a developing country might be difficult.

Score = 2

- Ease of storage, transportation and disposal of samples and tests

The Colilert tubes must be kept in a cool, dark and dry place, and the Petrifilm™ tests should be used within one month of purchase, or should be refrigerated until use (up to one year). This can be difficult, especially in areas where electricity is not always readily available, and if only a few tests per package are carried out and the rest must be stored in the refrigerator. The Colilert tubes are hard to transport and travel with since they take up more space and there is always the possibility that tubes may break. However, Petrifilms™ are easily transportable as long as the packages are not opened. Colilert tubes and Petrifilms™ can be disposed of by adding chlorine bleach the samples in the tube and Petrifilm, and by throwing the Colilert sample down the drain, and the Colilert tube and Petrifilm™ in the garbage.

Score = 3

- Ease of processing samples

Colilert tests are easy to process: 10 mL of sample must be poured into the Colilert tube that already contains the reagent, swirled, and then incubated. The Petrifilm™ test is more complicated: 1 mL of sample must be pipetted and dispensed onto the center of the pink agar. The top film must then be gently folded back into place, being careful not to trap air bubbles under the top film. Once the water has naturally spread out to fill the entire pink circle and has been setting for 1 to 2 minutes, the film can be placed between two pieces of cardboard and incubated. Many Sanitary Inspectors in the Philippines, and other professionals abroad, have had difficulty conducting EC-Kit tests, especially the Petrifilm™ test. Major problems include air being trapped under the top film, and sample overflowing to outer edges of pink agar.

Score = 3

- Incubation times

EC-Kit samples are incubated for 24 continuous hours.

Score = 4

- Use of electric incubator

One of the main features of the EC-Kit is the incubator belt, which allows samples to incubate using body heat alone. Therefore the EC-Kit does not require the use of an electric incubator

Score = 5

- Easy-to-read results

The Colilert test results record the presence or absence of *E.coli* and total coliform in a 10 mL sample volume. A yellow sample signifies the presence of total coliform and a

fluorescing (under UV light) sample signifies the presence of *E.coli*. Sanitary Inspectors often had trouble discerning a slightly yellow sample from a clear sample, and a fluorescing sample from a non-fluorescing sample. The 3M™ Petrifilm™ test results record the number of blue (*E.coli*) and red (total coliform) colonies with gas bubbles present in a 1 mL sample volume. Counting these colonies may be difficult, especially if many colonies are present and/or overlap, and if gas bubbles are small and hard to discern.
Score = 2

7.2.4. Practicality/Ease of Use Comparison

The following Table 7-6 summarizes the individual scores of the microbiological tests for each criterion and the total scores. This rating shows that HACH PathoScreen™ test is the most practical and easy to use (score of 30), whereas the EC-Kit test is the least practical or easy to use (score of 22).

Table 7-6. Practicality/Ease of Use Scores of New Microbiological Tests.

	H_2S test		Easygel	EC-Kit
	Laboratory	HACH		
1. Ease of training for test users: testers and readers	5	5	4	3
2. Ease of acquiring/making reagents	2	5	3	2
3. Ease of storage, transportation, and disposal of samples and tests	3	4	3	3
4. Ease of processing samples	5	5	4	3
5. Short incubation times	5	3	4	4
6. Use of electric incubator	5	5	5	5
7. Easy-to-read results	5	5	4	2
TOTAL	**30**	**32**	**27**	**22**

Therefore the HACH test is the most practical/easy to use field-based test, whereas the EC-Kit (Colilert and Petrifilm) is the least practical/easy to use field-based test.

8. Recommendations

8.1. Recommendations for individual tests

The following recommendations are made based on research using drinking water samples from improved and unimproved sources from Capiz Province, Philippines and from the Charles River in Cambridge, MA. We do not know how generalizable these recommendations are beyond these sources, and this is an important subject for future research.

8.1.1. Recommendations for the P/A Test for Improved Sources

Given the data evaluations and statistical analyses provided above, if a single P/A test were to be chosen to test improved sources, then the 20-mL H_2S test appears to be the best option. For unimproved sources, however, the 20-mL H_2S test results were merely as accurate as simply assuming that all unimproved sources were contaminated. As such, the 20-mL H_2S test can only be recommended as an appropriate test for improved sources.

The 20-mL H_2S test had the highest percentage of true results (84%), and somewhat low FP and FN values. Also, it had a higher percentage of FP's (10%) than FN's (6%), which is desirable since it signifies that the test errs on the side of caution. The 20-mL H_2S test was also shown to correlate to Standard Methods (Quanti-Tray® and membrane filtration) in a statistically significant way (through the chi-square test and Fisher's exact test), and the overall error associated with this test was relatively low at 20% for improved sources.

Furthermore, after the 10-mL H_2S test, the 20-mL H_2S test was the least expensive of the field-based tests, costing approximately $0.14 to $0.33 if purchased in the United States or Philippines, respectively. Lastly, the practicality/ease of use score of the 20-mL H_2S test was the second highest (after the 20-mL HACH test), meaning that it was one of the most practical field-based tests presented here.

8.1.2. Recommendations for the P/A Test for Unimproved Sources

Given the data evaluations and statistical analyses provided above, if a single P/A test were to be chosen to test unimproved sources, then the 10-mL pre-dispensed P/A Colilert test seems like the best option. However, for improved sources, the Colilert test results were less accurate than the 20-mL H_2S test. As such, the Colilert test is recommended as an appropriate test for unimproved sources only.

The Colilert test has a highest percentage of TR (83%), and somewhat low FP and FN values. The Colilert test was also shown to correlate to Standard Methods (Quanti-Tray® and membrane filtration) in a statistically significant way (through the chi-square test and Fisher's exact test), and the overall error associated with this test is relatively low at 5% for unimproved sources.

However, the 10-mL pre-dispensed P/A Colilert test is relatively expensive and can cost up to $3.20/test if only 10 samples are purchased, (Table 7-4). The average cost per test could be lowered if Colilert tests were purchased in bulk. The Colilert test is also practical/easy to use as a simple P/A test, although special care needs to be taken when training individuals on interpreting the tests, namely for color change and fluorescence.

8.1.3. Recommendation for Enumerative test for Improved Sources

Given the data and statistical analyses provided above, if a single enumerative test were to be chosen to test improved sources, then the Easygel® test appears to be the best option. In fact, for unimproved sources, Easygel® test results were less accurate than simply assuming that all unimproved sources were contaminated. As such, the Easygel® test can only be recommended as an appropriate test for improved sources only.

Easygel® had the highest percentage of TR (81%), and very few FP's (1%) and high FN's (17%). It was also shown to correlate to Standard Methods (Quanti-Tray® and membrane filtration) in a statistically significant way (through the chi-square test and Fisher's exact test), and the overall error associated with this test is relatively low at 18% for combined unimproved and improved sources. The error for improved sources is high (25%). However, this is probably due to small sample size (n=14 and n=28), which was proven with Fisher's exact test to be statistically insignificant (p-value= 0.16).

Furthermore, Easygel® is relatively inexpensive and is currently priced at approximately $1.63 (if more than 10 Easygel® sets are purchased) if purchased in the United States. Lastly, the practicality/ease of use score of Easygel® was similar to the score of the H_2S test. The main differences in score are due to the Easygel® being an enumerative test, which means that it is slightly more complicated to train new test users and to interpret test results. Another advantage of the Easygel® test is that the testing procedure may be modified to test for sample volumes ranging from 0.5 mL to 5 mL, which is an interesting option if the sample to be tested is expected to be highly contaminated (where a smaller sample volume would be used) or if the sample to be tested is expected to be slightly contaminant (where a larger sample volume would be used).

8.1.4. Recommendation for Enumerative test for Unimproved Sources

The enumerative tests assessed in the study were Easygel® and Petrifilm™. For unimproved sources, both tests individually yielded results that were less accurate than simply assuming that all unimproved sources were contaminated (λ=-100% and λ=-133% for Easygel® and Petrifilm™, respectively). Therefore, it is recommended that instead of using an enumerative test to assess the water quality of unimproved sources, a more accurate P/A test be used (i.e. Colilert); or that otherwise no tests (P/A or enumerative) be performed and that the an unimproved water source is automatically assumed to be contaminated.

8.2. Recommendations for Test Combinations

Given the data evaluations and statistical analyses provided above, of the test combinations presented here, the combination of the 20-mL H_2S test + Easygel® test appears to be the best option. Although any of the H_2S tests presented here (10-mL, 20-mL, 100-mL and 20-mL HACH) in combination with Easygel® yielded the same error and proportional reduction in error (0% and 100%, respectively) (Table 6-42), the 20-mL H_2S test + Easygel® combination was chosen as the best option based on the accuracies of the individual H_2S tests, their cost and practicality/ease of use.

However, it must be noted that the both the 20-mL H_2S tests and Easygel® were performed for 23 samples only (four unimproved sources and 19 improved sources), whereas the EC-Kit test results presented here were performed for over 150 samples.

The 20-mL H_2S test and Easygel® together would cost approximately $1.77 ($0.14 for the 20-mL H2S test reagent only and $1.63 for Easygel®), if purchased in the United States. The advantages of these two tests in terms of practicality/ease of use were discussed in 8.1.1 and 8.1.3.

8.3. Recommendations for Future Studies

8.3.1. Verification of Easygel® as a Single Enumerative Test, and 20-mL H_2S Test + Easygel® Combination

Although the 20-mL H_2S test + Easygel® proved to be the best field-based test combination, further verification of the Easygel® and the 20-mL H_2S test and Easygel® combination still needs to be performed. Therefore a large-scale (150+ samples) verification program should be undertaken in order to determine the accuracy of the Easygel® test, as a single enumerative test, and the 20-mL H_2S test and Easygel® combination, as potential replacement or additional tests to the current EC-Kit tests (Colilert and Petrifilm™) in a new field-based testing kit. The verification should be undertaken in conjunction with an enumerative Standard Methods test such as Quanti-Tray® or membrane filtration, along the lines of this thesis research. In addition, other water sources beyond Capiz Province, Philippines and Cambridge, MA, should be tested to confirm whether results reported in this thesis are generalizable.

8.3.2. Study concerning the suitability of Petrifilm™ and Easygel® as field-based tests in tropical countries

One of the main surprises of this study is that the Petrifilm™ test (which was shown to be a reliable, easy-to-use microbiological test in the United States (Vaila, Morganb, Merinoc, Gonzales, Millerb, & Ram, 2002)) did not perform as well as expected. In fact, it only yielded 67% TR (Table 6-26). This could potentially be due to the Petrifilm™ test being developed as a microbiological test for temperate countries alone, or where the agar media may not be suitable in tropical countries. Therefore, a study, which examines the suitability of the Petrifilm™ and Easygel® tests for tropical countries, would help shed light on the appropriateness of these tests in developing countries.

8.3.3. Verification of the 20-mL H_2S test as a MPN test

The 20-mL H_2S test has been used as a MPN test (HACH, 2000). Like the Quanti-Tray®, the results of the five-tube 20-mL H_2S tests could be used to determine an approximate MPN count: from smaller than 1.1 MPN/100 mL (five tubes indicate absence of H_2S-producing bacteria) to greater than 8 MPN/100 mL (five tubes indicate presence of H_2S-producing bacteria) (HACH, 2000). The use of the 20-mL H_2S test as an MPN test was not investigated in this research. However, given the accuracy of the 20-mL H_2S test as a single P/A test, and the cost and practicality/ease of use of the 20-mL H_2S test, it would be worthwhile to conduct a study that verifies the accuracy of the 20-mL H_2S test as an MPN test. The five-tube 20-mL H_2S test would be less expensive than the Easygel® test ($0.14 x 5= $0.70 for five 20-mL H_2S tests vs. $2.13 for Easygel), and could perhaps yield more accurate results. Furthermore, the 5-tube 20-mL H_2S tests would also be easy to conduct, test results would be easy to read, and test users could be trained easily and rapidly.

9. Conclusion

The general objective of this study was to verify and assess the suitability of four new, low-cost, microbiological field-based tests to be used for drinking water quality testing in developing countries. More specifically, the study looked at the laboratory-made P/A H_2S test (for 10-, 20- and 100-mL sample volume) originated by Manja, Maurya, and Rao (1982), the HACH PathoScreen P/A H_2S test (20-mL sample volume) and the enumerative Easygel® test. The study compared these tests to those currently used as part of a newly-developed testing kit: the EC-Kit, comprised of the 10-mL P/A Colilert test and the enumerative Petrifilm™ test[1]. The study also assessed H_2S-producing bacteria as a valid indicator of fecal contamination.

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, MA in April 2010. In total, 203 samples were tested using the 10- and 20-mL laboratory-made H_2S reagent; 202 samples were tested using the 100-mL laboratory-made H_2S reagent; 203 samples were tested using the HACH PathoScreen™ 20-mL H_2S test; 83 samples were tested using the Coliscan® plus Easygel®; and 218 samples were tested using Colilert and Petrifilm™.

The different tests were verified and compared based on accuracy, cost and practicality/ease of use. Accuracy was measured by comparing the test results to test results obtained using Standard Methods tests (Quanti-Tray® and membrane filtration). Cost (both fixed and variable) was for reagents, tests, vials and bottles for purchase in the United States and the Philippines. Practicality/ease of use was measured by comparing the way each test scored on a set of 7 criteria: (1) ease of training new test users, (2) ease of acquiring or making reagents, (3) ease of storage, transportation and disposal of samples, (4) ease of processing samples, (5) short incubation times, (6) use of electric incubator, (7) easy to read results.

[1] The EC-Kit was verified in the MIT M.Eng. thesis of Chuang (2010).

The tests were looked at as single P/A or enumerative tests, and as a combination of tests (i.e. one P/A test and one enumerative test) to determine the best testing combination. Based on the criteria listed above, the study recommended the use of the laboratory-made 20-mL H_2S test as a single P/A test for testing improved water sources, and the use of the Colilert test as a single P/A test for testing unimproved water sources. The use of the Easygel® test as a single enumerative test was recommended for testing improved water sources only, and the use of the enumerative tests presented in this thesis (Easygel® and Petrifilm™) was discouraged for unimproved sources. Lastly, the combination of the 20-mL H_2S test and Easygel® combination was recommended for field-based microbiological drinking water quality testing for all water sources.

Given the statistical analyses presented above, H_2S-producing bacteria was found to be a valid indicator of fecal contamination for improved sources alone. However, further testing is recommended to ensure that the H_2S-producing bacteria meet all the WHO requirements for an ideal indicator of fecal contamination for both improved and unimproved water sources.

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