Comparison of EC-Kit with Quanti-Tray®: Testing, Verification, and Drinking Water Quality Mapping in Capiz Province, Philippines

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ABSTRACT

This thesis accomplishes three tasks. First, it verifies the EC-Kit under different water source conditions by comparing it to a laboratory standard method, the IDEXX Quanti-Tray®. The EC-Kit is a simple, inexpensive field test kit that contains complementary tests for *Escherichia coli* and total coliform: the Colilert® 10-milliliter presence/absence test and $3M^{TM}$'s PetrifilmTM test. This work was executed by analyzing 521 water samples collected in Capiz Province, Philippines as well as 40 water samples from the Charles River in Cambridge, Massachusetts. Second, it determines the risk level for drinking water sources according to *E.coli* and total coliform levels in Capiz Province for difference locations and source types. Third, this study contributes to an ongoing mapping project, aimed at creating an interactive, searchable map of water quality results from EC-Kit and Quanti-Tray®.

The results of the study reveal that each component of EC-Kit and the entire kit itself is correlated to Quanti-Tray® in a statistically significant way. Moreover, from the calculations of error and proportional reduction in error for unimproved/improved water sources, it is possible to make better predictions with just the use of the Colilert® test, but not just the use of the PetrifilmTM. This is because the detection limits for PetrifilmTM are an order of magnitude higher than Colilert®, namely PetrifilmTM colony counts of 1-10/1 mL sample results fall within the High and colony counts of 10-100/1 mL of sample fall within the Very High risk level categories, whereas positive Colilert® results fall within the Intermediate, High, and Very High risk level categories. Most importantly, the EC-Kit allows for the best reduction in error, with a proportional reduction in error of 63% for unimproved water sources and 60% for improved water sources. This finding is significant because it means that a simple, inexpensive field kit can change our understanding of the safety of drinking water compared to simply knowing the United Nations infrastructure designation of improved versus unimproved water sources. Furthermore, the statistical analyses revealed that while the EC-Kit does not exactly match the Quanti-Tray® results, it still provides useful information for assessing at-risk water sources.

Thesis Supervisor: Susan Murcott

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List of Abbreviations

3M	Minnesota Mining and Manufacturing Company
APHA	American Public Health Association
AWWA	American Water Works Association
BCIG	5-bromo-4-chloro-3-indolyl ß-D-glucuronide
cfu	colony forming units
СР	ColiPlate
DPH	Director of Public Health
E.coli	Escherichia coli
EC	Escherichia coli
GPS	Global positioning system
km	kilometers
Lambda (λ)	Proportional reduction in error
MF	Membrane Filtration
MIT	Massachusetts Institute of Technology
mL	milliliters
MPN	Most probable number
NGO	Non-governmental organization
NSCB	National Statistical Coordination Board
P/A	Presence/absence
РНО	Provincial Health Office
PNSDW	Philippines National Standards for Drinking Water
SI	Sanitary Inspector
TC	Total coliform
TM	Trademark
TNTC	Too numerous to count
UN	United Nations
UNICEF	United Nations Children's Fund
UV	Ultraviolet
WEF	Water Environment Federation
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

1.1 Background

Water is a basic human need for health and survival. The United Nations (U.N.) describes water as "indispensable for leading a life of human dignity" and "a prerequisite for the realization of other human rights." This right to water "entitles everyone to sufficient, safe, acceptable, physically accessible, and affordable water for personal and domestic uses" (United Nations, 2009). Still, thirteen percent of the world's population, or 884 million people, lack access to an improved drinking water source (WHO/UNICEF, 2010). Every year, at least 1.8 million people die from diarrheal diseases related to unsafe water, sanitation, and hygiene. Furthermore, the majority is children under the age of five years old (WHO/UNICEF, 2010).

In response, the U.N. has developed the Millennium Development Goal 7 Target 3 aimed to halve the proportion of people without sustainable access to adequate water and sanitation by the year 2015 (United Nations, 2000). The indicators used for these numbers include the proportion of the population that uses an improved drinking water source, and the proportion that uses an improved sanitation facility (United Nations, 2000). The ideal solution to the problem of unsafe water and subsequent disease is to provide reliable access to a safe drinking water supply. According to the U.N., access to drinking water means that the source is less than one kilometer away from its place of use, and that it is possible to reliably obtain at least 20 liters of water per member of a household per day. The U.N. also defines safe drinking water as water with microbial, chemical, and physical characteristics that meet World Health Organization (WHO) guidelines or national standards on drinking water quality (United Nations, 2000).

This raises the important issue of the performance of water quality testing in order to determine the safety of drinking water. Yet, the assessment of safe drinking water by means of microbiological indicator testing is frequently expensive, particularly in developing countries. This project aims to verify an inexpensive and simple water quality assessment tool, the *Escherichia coli*-Kit (EC-Kit) Portable Microbiology Laboratory. Additionally, this project will be selectively testing the water quality in Capiz Province, for the purposes of determining the locations of unsafe drinking water supplies, so that corrective action may be taken.

1.2 Scope of Current Work

The current study has the following objectives:

- 1. To verify the field method EC-Kit under different water source conditions by comparing it to a laboratory standard method (IDEXX Quanti-Tray®). The results of this work are found in Chapters 4, 5, 6 and 7.
- To conduct a detailed, in-depth controlled experiment of Charles River Water from Cambridge, Massachusetts with multiple sample dilutions, duplicates, and blanks. The results of this work are in Chapters 4 and 5.
- To determine the improvement in making predictions of drinking water quality compared to simply knowing the United Nations infrastructures designation of improved versus unimproved water sources. The results of this work are found in Chapter 6.
- 4. To determine the water quality and risk level for drinking water sources according to *Escherichia coli* and total coliform tests in Capiz Province for different locations and source types. The results of this work are found in Chapters 3, 4, and 5.
- 5. To create a map of the water quality results from EC-Kit and Quanti-Tray[®]. The results of this work are found in Chapter 6.

1.3 Overview of the Philippines

The Philippines is an archipelago composed of over 7,000 islands and is located in Southeast Asia, between the Philippine Sea, Celebes Sea and the South China Sea. It is a mountainous country with low-lying reaches along the coastline. The Philippines have a total land area of approximately 300,000 km² and an extensive coastline of over 36,000 km. There is a tropical marine climate and two monsoon seasons: the dry, northeast monsoon from November to April, and the wet, southwest monsoon from May to October. The country is usually subject to 15 typhoons per year and five to six cyclones, which impacts both water and land resources (Central Intelligence Agency, 2010).



Figure 1: Map of the Philippines (Central Intelligence Agency, 2010).

A census conducted in July 2009 estimated the population at almost 98 million, making it the 12th most populated country in the world. The Philippines has an infant mortality rate of 24 per 1,000 and the life expectancy is 71 years. Despite the long life expectancy, the risk of infectious disease is high in the country. Food and waterborne diseases such as bacterial diarrhea, hepatitis A and typhoid fever abound. The high population density, increasing level of urbanization (65%, 3% growth rate) and the tropical marine climate exacerbate food and waterborne diseases (Central Intelligence Agency, 2010).

The country is populated by a variety of ethnic groups, including Tagalog, Cebuano, Llocano, Bisaya/Binisaya, Hiligaynon Llonggo, Bikal, Waray and others; in total there are over one hundred groups (The Official Government Portal of the Republic of the Philippines, 2010). The vast majority are Christian, with 91.5% estimated by the 2000 census (81% Roman Catholic). The Philippines is a Democratic Republic and is divided into three geographic areas - Luzon, Visayas, and Mindanao. There are a total of 81 provinces, 136 cities, 1,494 municipalities and 41,995 *barangays* (a geographical area within a city or municipality comprised of less than 1,000 inhabitants), which are the smallest organizational unit in the Philippine political system. The capital city is Manila, which is located in Luzon. The current President, President Gloria Macapagal-Arroyo, has been in power since 2001 and the next election is set for May 2010.

Philippines economy is primarily based on service (commerce and government), industry and agriculture; with a rough breakdown of >50%, 30%, <20%, respectively (United States Department of State, 2010). Arable land and permanent crops account for approximately 35% of the total land use, and a total of 15,000 km² is irrigated land (in 2003). The major agriculture products are: rice, sugarcane, coconut, corn, bananas, cassavas, pineapples, mangoes, pork, eggs, beef, and fish. Industry includes electronics assembly, garments, footwear, pharmaceuticals, chemicals, wood products, food processing, petroleum refining, and fishing. The GDP growth rate in 2008 was 3.8% and the GDP per capita as of 2008 has been reported by the CIA as \$3300 and by the United States Department of State as \$1,841 (United States Department of State, 2010). Forty-one percent of Filipinos continue to live in rural areas and 47% of rural families continue to live below the nationally defined poverty line in 2000, compared with 20% of urban families (World Bank, 2006).

1.4 Water Use in the Philippines

The total renewable water resources in the Philippines in 1999 were estimated to be 479 km^3 (Central Intelligence Agency, 2010). With freshwater withdrawals in 2000 estimated at approximately 29 km^3 per year; with a breakdown of 17%, 9% and 74% for domestic, industrial and agricultural uses, respectively. Agriculture is a significant draw on the freshwater resources. Approximately 5% (15,500 km^2 / 300,000 km^2) of land area in the Philippines was irrigated in 2003. The use of irrigation is increasing, with the threats of climate change and El Niño causing

droughts and below average rainfall in certain areas in recent years. In fact, the President has recently called for early completion of a major national irrigation project in light of these facts (The Official Government Portal of the Republic of the Philippines, 2010). 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.

1.5 Drinking Water Regulations in the Philippines

Chapter II (Water Supply), Section 9 of the Code on Sanitation of the Philippines states that "Standards for drinking water and their microbiological and chemical examinations, together with the evaluation of results, shall conform to the criteria set by the National Drinking Water Standards" (The Republic of the Philippines Department of Health, 1976). In 2007, the Philippines Department of Health formulated standards for drinking water, aiming to minimize risk and therefore prevent health repercussions from exposure to impurities in water. The standards set in the Philippines National Standards for Drinking Water (PNSDW) 2007 are based on guidelines or criteria recommended by international institutions like the WHO. The Philippines National Standards for Drinking Water (PNSDW 2007) addressed water quality issues by setting more comprehensive parameters, advocating a surveillance system, and prioritizing the parameters that need to be monitored (The Republic of Philippines Department of Health, 2007).

The WHO Joint Monitoring Programme for Water Supply and Sanitation has been assembling statistics on drinking water and sanitation coverage since 1990. Since 2000, the Joint Monitoring Programme has classified water sources as "improved" or "unimproved" (WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation, 2005). The classification of "improved" and "unimproved" water technologies are shown in Table 1.

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

 Table 1: World Health Organization Water Source Classification

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

There are four water source categories used in the Philippines, which are defined in Table 2. Doubtful sources are equivalent to the U.N. "unimproved" category and Level 1, Level 2 and Level 3 are equivalent to the U.N. as "improved" category.

U.N. Designation	Philippines Designation	Source Types	Capiz Province
Unimproved	Doubtful	Unimproved springs, open dugwells or wells that need priming, surface water, or rainwater collectors	8%
	Level 1	Stand-alone point sources, including shallow wells,	
Improved Level 2		Piped water supply with communal water points, from	92%
	Level 3	Piped water supply with private water points, such as a	

Table 2. Levels of Drinking Water Sources in the Philippines

According to the National Statistical Coordination Board (NSCB) of the Philippines, as of 2000, 119,000 households in Capiz have access to an improved drinking water supply. In other words, approximately 92% of the population of Capiz had access to improved drinking water source in 2000 (total number of households in Capiz was approximately 129,000 in 2000) (NSO, 2002). According to the Philippines government's regulatory definitions, "improved"

water sources include three levels: Level 1 consists of point sources, Level 2 consists of communal faucet systems, and Level 3 consists of piping systems with individual household connections. The unimproved water sources, also called "doubtful sources," consist of open dug wells, unimproved springs, rainwater and surface water sources.

1.6 Overview of Capiz Province, Philippines

Capiz Province is located on the northeastern part of Panay Island, which is located in the Western Visayas. It has a land area of approximately 2,600 km² and has roughly 80 km of coastline. It is a major center for the aquamarine industry in the country, as well as a center for tourism and agriculture. The population has been estimated in to be about 700,000. It is composed of 16 municipalities, 1 city (Roxas City) and 473 *barangays* (villages).

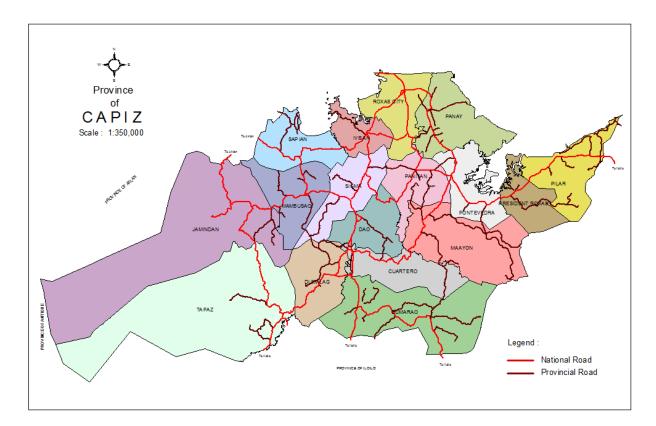


Figure 2: Municipalities of Capiz Province (Capiz Provincial Health Office).

The capital city, Roxas City, is located along the northern edge of the province and has a population of approximately 132,000. Similar to the rest of the province, fishing and farming are

the major economic activities; which together use just over 50% of the total land area. The dominant agricultural crop is rice, with over 38 km² of land used for rice fields. Other major crops include coconuts, bananas, watermelons, leafy vegetables, mungo, various citrus crops and mango. Both freshwater and brackish water aquaculture is common, as the swampy coastline lends itself well to fishpond development. In fact, over 840 km² are used for brackish fishpond development. Marine fishing and livestock production are also major industries in the area. As the only urban area in the province, Roxas City is a center of trade and commerce, and as a result is becoming increasingly industrialized and commercialized.

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 the water quality testing in the province. The main PHO participants in this project included Dr. Jarvis Punsalan, MD, 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, and United Nations Children's Fund (UNICEF) to set up a water quality testing laboratory at Roxas Memorial Hospital, in Roxas City, which would test for microbiological contamination. He contacted Susan Murcott, Senior Lecturer at the Massachusetts Institute of Technology (MIT), for advice on the types of microbiological drinking water quality tests to conduct, and she recommended two types of tests: 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 35 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) Colilert® and the enumerative test: $3M^{TM}$ PetrifilmTM. The innovation of combining these two tests in the EC-Kit was the idea of Dr.

Robert Metcalf, one of the original founders of solar cookers international 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 the EC-Kit, which included all the items, including a waist belt incubator, and other materials needed in order to perform and interpret the tests. Susan Murcott introduced the technology to the Non-Governmental Organization (NGO) "A Single Drop", and introduced the director of that NGO to Robert Metcalf, after which they brought the technology to the Philippines.

During 2009, Capiz's PHO purchased EC-Kits and Quanti-Tray® tests. An incubator, ultraviolet (UV) light and Quanti-Tray® sealer were also purchased in order to conduct the Quanti-Tray® tests. In May 2009, "A 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 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 for 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.

CHAPTER 2: LITERATURE REVIEW

2.1 Indicator Microorganisms

There are a large variety of bacteria, parasites, and viruses that can cause illness when humans ingest them in drinking water. However, testing drinking water for all possible diseasecausing agents would be difficult, time-consuming, and expensive. Instead, monitoring drinking water quality relies on the detection of indicator organisms. According to the WHO (World Health Organization, 2006), ideal indicator organisms should:

- 1. be universally present in feces of humans and animals in large numbers;
- 2. not multiply in natural waters;
- 3. persist in water in a similar manner to fecal pathogens;
- 4. be present in higher numbers than fecal pathogens;
- 5. respond to treatment processes in a similar fashion to fecal pathogens; and
- 6. be readily detected by simple, inexpensive methods.

While there is no perfect indicator organism, *Escherichia coli* is considered the most reliable indicator of fecal contamination (Doyle & Erickson, 2006). *E. coli* and thermotolerant coliforms are a subset of the total coliform group. Total coliform bacteria are defined as aerobic and facultatively anaerobic, gram-negative, non-spore forming, rod-shaped bacteria that produce gas upon lactose fermentation in prescribed culture media after 24 hours of incubation at 35°C (World Health Organization, 2006). Total coliform counts are used to monitor water treatment, but total coliforms include both fecal and environmental species. Therefore, to avoid the limitations of total coliforms, *E. coli* and thermotolerant coliforms are widely accepted as good indicators for fecal contamination.

2.2 Overview of Microbial Assay Methods

For analyzing drinking water quality, particularly in routine public water supply examination, the object is to determine the efficiency of treatment plant operation, the integrity of the distribution system, and to screen for the presence of fecal contamination (APHA, WEF, AWWA, 2005). The four Standard Methods (SM) most commonly used to identify coliforms in

water include multiple-tube fermentation (SM#9221A), presence/absence (SM #9221D), membrane filtration (SM #9222), and enzyme substrate (chromogenic) (SM #9223).

In the multiple-tube fermentation test, multiple tubes are used in the fermentation, and the Most Probable Number (MPN) of organisms present is reported. When utilizing multiple-tube fermentation, the precision of each test depends on the number of tubes used.

The presence-absence (P-A) test for coliforms is a modified, simpler version of the multiple-tube fermentation test. The P-A test operates under the theory that no coliforms should be present in 100 mL of a drinking water sample, and so it is possible to conduct a test using one test volume. The P-A test is intended for use on routine samples collected from distribution systems or water treatment plants. However, in the event of a positive result for coliforms (presence), it is advisable to determine coliform densities in repeat samples and/or other tests (APHA, WEF, AWWA, 2005).

The membrane filter (MF) technique, as described in Standard Methods for Examination of Water and Wastewater, 21st edition, is routinely used worldwide to quantify density of coliforms and *E. coli* in water and wastewater (Lifshitz & Joshi, 1997). The MF technique is highly reproducible, can test relatively large sample volumes, and yields numerical results faster than multiple-tube fermentation. However, the MF technique has limitations in testing waters with high turbidity or large numbers of noncoliform bacteria because the presence of algae, particulates, or other interfering material may not permit testing of sufficient sample volume to yield significant results (APHA, WEF, AWWA, 2005).

The enzyme substrate test is recommended for the analysis of drinking and source water samples (APHA, WEF, AWWA, 2005), but it is emphasized for laboratories using this text to conduct parallel quantitative testing (including seasonal variations with one of the standard coliform tests to assess the effectiveness of the test for the specific water type, particularly when testing source waters. The enzyme substrate test is not recommended for presumptive coliform cultures or membrane filter colonies because it may lead to false positives.

2.3 Quanti-Tray® and Quanti-Tray®/2000

The IDEXX Quanti-Tray® and Quanti-Tray®/2000 are enzyme substrate coliform tests that utilize semi-automated quantification methods based on the Standard Methods Most

Probable Number (MPN) model, which provides the MPN of colony forming units (cfu). The tests have been approved in over 35 countries worldwide (IDEXX, 2009). The trays provide bacterial counts of up to 200.5 MPN per 100 mL of sample (or 2419 MPN per 100 mL of sample for Quanti-Tray®/2000).

The Quanti-Tray® is easy, rapid, and accurate. There is no colony counting required, no dilutions needed for counts up to 2,419, and no media preparation. The Quanti-Tray® detects down to 1 cfu per 100 mL of sample, and has better 95% confidence limits than multiple tube fermentation or membrane filtration (Thermalindo, 2007). However, the cost of equipment and supplies for Quanti-Tray® is expensive, particularly in developing countries.

2.4 EC-Kit

The EC-Kit is a simple, inexpensive field test kit that contains two complementary tests for *E. coli*: the Colilert® 10 mL presence/absence test and $3M^{TM}$'s PetrifilmTM test. The kit also includes sterile sampling bags, a sterile 3.5-mL pipette, an ultraviolet light with batteries, cardboard squares, rubber bands, and a waist-belt incubator. The 10 mL pre-dispensed Colilert® test indicates presence/absence of coliform bacteria, and specifically whether *E. coli* is present in the 10 mL of water tested. The PetrifilmTM test provides a quantitative count of total coliform bacteria and *E. coli* present in the volume sampled.

2.5 Studies on Colilert® Reagent

Olson et al. compared Colilert[®] and ColiQuik¹ systems in presence-absence format against the Standard Methods Membrane Filtration (MF) technique for total coliform detection, and observed a greater than 94.8% agreement in two-way comparisons (Colilert[®] with MF, ColiQuik with MF) (Olson, Clark, Milner, Stewart, & Wolfe, 1991). When laboratory and field inoculation methods were compared for Colilert[®], more than 98% agreement was obtained. Due to the presence of false negatives in the study, the researchers indicated the importance of using field data and not spiked water samples in future studies.

¹ ColiQuik is suitable for testing salt water, chemically treated water and wash from meat, fish and vegetable preparation. USA Patent No. 6051394. <u>http://www.b2ptesting.com.au/Coliquik%20Pack%20Insert_LATEST.pdf</u> (B2P Testing, Auckland, New Zealand)

Lifshitz and Joshi compared MF with the new ColiPlate (CP) kit² on water samples collected from water treatment plants' intakes and water pollution control plants' final effluents (Lifshitz & Joshi, 1997). While there was a strong correlation observed for enumerating *E.coli* ($R^2 = 0.95$, Figure 3), considerably higher counts were determined by CP than with MF. The authors cite biological characteristics of cells, methods of enumeration, and failure of the MF technique to recover injured/weakened cells as possible explanations for the discrepancy. The overall conclusion, however, is that the CP test is just as or more sensitive for enumerating coliforms and *E. coli* in water than the MF test.

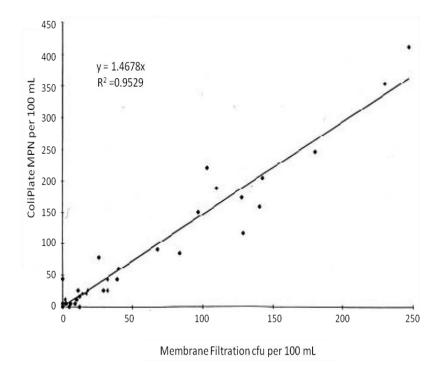


Figure 3: Comparison between membrane filter and ColiPlate methods for enumerating *E.coli* in natural water samples (Lifshitz & Joshi, 1997).

² ColiPlateTM test quantifies density of target bacteria, coliforms and *E. coli*, ranging from 5 to 5,000 colony forming-units (cfu) per 100 mL sample, without dilutions. It utilizes defined substrate technology (using 4-methylumbelliferyl-D-glucuronide (MUG) substrate) for the purpose of enumerating *E. coli* as an indicator of fecal contamination. Manufactured by Bluewater Bioscience Inc.

Fricker et al. compared the newer form of Colilert®, Colilert®-18, to MF (Fricker, Illingworth, & Fricker, 1997). Colilert®-18 provides results within 18 hours instead of the traditional 24-hour Colilert® test. Their study showed that there was no significant difference between the two Colilert® and Colilert®-18 forms of the product. Colilert®-18 showed similar results to membrane filtration. Therefore, both formulations of the Colilert® reagent, Colilert® and Colilert®-18, were suitable alternatives to the membrane filtration method for bacteriological monitoring of drinking water quality (Fricker, Illingworth, & Fricker, 1997).

Years later, in 2003, Chao et al. conducted an edifying study on the accuracy of Colilert®-18 as a test for coliforms and *E. coli*. It was found that Colilert®-18 produced a low false-negative rate, and would serve well as a technique for monitoring subtropical freshwaters. The authors point out that for some countries, total coliform number still serves as the sole indicator microorganism, and Colilert®-18 tends to give higher total coliform counts than the traditional methods, such as membrane filtration. Otherwise, it was also concluded that Colilert®-18 is satisfactory for rapid screening for fecal contamination in subtropical freshwater (Chao, Chao, & Chao, 2004).

From these studies on Colilert® reagent and given what is known about Quanti-Tray®, is clear that Colilert® is comparable to Colilert®-18, and that both are suitable alternatives to the membrane filtration Standard Method. Colilert®-18 produces a low false negative rate, and may even be more sensitive for enumerating coliforms. Thus, Quanti-Tray® and Colilert® (or Colilert®-18) are apt for use as the standard water quality test to which the EC-Kit will be compared for verification.

2.6 Studies on 3M PetrifilmTM

In a study conducted by Vail et al., PetrifilmTM total coliform count plates (Manufactured by $3M^{TM}$, Minneapolis, Minnesota), previously used for enumerating *E. coli* in food, were tested for monitoring *E. coli* in environmental waters (Vail, Morgan, Merino, Gonzales, Miller, & Ram, 2003). The study compared enumeration of *E. coli* in water samples using PetrifilmTM to three commonly used commercially available tests: membrane filtration using mColiBlue media, mTEC media, and Colilert® Quanti-Tray® (Figure 4). The data was normalized to 100 mL and transformed with log[cfu/100 +10] prior to linear regression. It was concluded that PetrifilmTM

results were highly correlated (R>0.9) and equivalent to mColiBlue, mTEC, and Colilert® Quanti-Tray® tests (Comparison with m-Coliblue, R=0.995, p<0.001, comparison with mTEC method, R=0.93, p<0.001, comparison with Colilert®-18/IDEXX Quanti-Tray® method, R=0.935, p<0.001). However, while PetrifilmTM plates appear to be useful as a first step in obtaining environmental *E. coli* isolates, the researchers emphasized the need for due care in evaluating the presence of gas bubbles to determine rates of false positives and false negatives for validation.

This conclusion is particularly important in EC-Kit testing and verification because of the difficulties in counting PetrifilmTM results. Counting colonies with gas bubbles needs to be enforced in EC-Kit training to maintain the high correlation between PetrifilmTM and Quanti-Tray®.

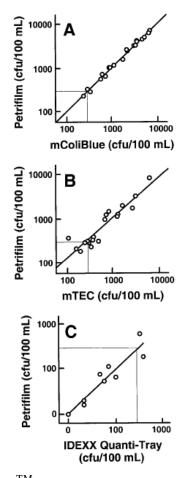


Figure 4: Comparisons of PetrifilmTM to three standard methods (Vail, Morgan, Merino, Gonzales, Miller, & Ram, 2003).

Schraft & Watterworth (2005) compared PetrifilmTM with standard plating procedures (mFC-agar plates) on naturally contaminated water samples for enumeration of heterotrophs, fecal coliforms, and *E. coli* in water (Schraft & Watterworth, 2005). On mFC agar, counts for typical colonies were 2 \log_{10} cfu higher than the actual confirmed counts (confirmed via biochemical identification using Standard Procedures for Water Analysis, sections 9221B, 9221E, 9225A), whereas PetrifilmTM EC plates were almost identical to confirmed colony counts for both fecal coliforms and *E. coli* (Figure 5). Thus, it was found that PetrifilmTM plates seemed more selective for fecal coliforms and *E. coli*.

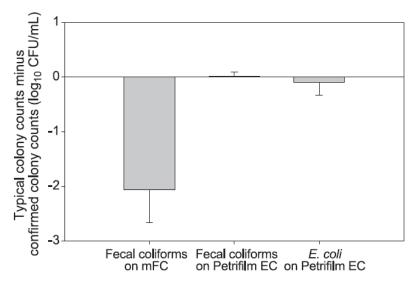


Figure 5: Difference between typical colony counts and confirmed colony counts obtained on mFC agar and PetrifilmTM EC plates (Schraft & Watterworth, 2005).

This study is one of the few studies evaluating the use of PetriflmTM as a test for water quality. PetrifilmTM is more selective for fecal coliforms and *E.coli*, meaning that it is a better "match" to the confirmed counts than the standard plating procedure using mFC-agar. However, there have been no studies evaluating the use of PetrifilmTM as a field test to be used outside of the laboratory, as this study aims to do.

CHAPTER 3: RESEARCH AND SAMPLING DESIGN

3.1 Overview of Research and Sampling Design

Capiz Provincial Health Officer Dr. Jarvis Punsalan (MD, MPH), and Sanitary Inspector Jane Delos Reyes, commenced the 1,000 test set program covering 16 municipalities and Roxas City in December 2009. The author travelled to and worked in Capiz for approximately 22 working days, beginning on January 7, 2010, together with three other Master of Engineering teammates and their project advisor. The study design was prepared by Punsalan and Reyes, in collaboration with Susan Murcott of the Massachusetts Institute of Technology (MIT), Tom Mahin of the Massachusetts Department of Environmental Protection, and our four-person Masters of Engineering student team. The overarching objective for all collaborators was to (i) Determine, for the first time, the water quality in the 16 municipalities and Roxas City, (ii) Compare two different test methods: Quanti-Tray® and EC-Kit to determine if the EC-Kit is a reliable field test method for local application beyond this study, (iii) Evaluate the chlorine residual levels in Roxas City to determine if they met the PNSDW regulatory standards and (iv) Based on sanitary inspector and stakeholder interviews and community assessments, make recommendations on how to increase the safety of drinking water in Capiz Province.

The number of villages or sampling zones for each municipality was computed based on the following criteria (Table 3): a ratio of 1 sampling zone for every 5,000 population (e.g. a municipality with a population of 30,000 would have 6 sampling zones selected). Zones were distributed according to ratio of water sources accessed by the residents of the particular municipality.

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

• For each selected zone having doubtful sources, five of these sources were randomly selected and tested.

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

The only exception to the aforementioned study design was the Level 3 water supply for Roxas City. Since all of Roxas City has a piped, chlorinated water supply, this was tested separately using chlorine residual testing instead of the costlier bacteriological testing.

									Dou	ıbtful	Lev	vel 1	Lev	vel 2	Lev	vel 3	
Municipality	NUMBER OF VILLAGES	WITH DOUBTFUL ACCESS? (Y/N)	WITH LEVEL 1 ACCESS? (Y/N)	WITH LEVEL 2 ACCESS? (Y/N)	WITH LEVEL 3 ACCESS? (Y/N)	TOTAL HOUSEHOLDS (2008 DATA)	ESTIMATED POPULATION	APPROXIMATE NUMBER OF VILLAGES TO BE SELECTED AS SAMPLING ZONES	# OF VILLAGES	# OF SAMPLING SOURCES	TOTAL SAMPLES						
Cuartero	22	Y	Y	Y	Ν	5340	28733	6	1	5	4	20	1	5	0	0	30
Dao	20	Y	Y	Ν	Ν	6071	36233	7	1	5	6	25	0	0	0	0	30
Dumalag	19	Y	Y	Y	Y	5989	30669	6	1	5	3	15	0	0	2	10	30
Dumarao	33	Y	Y	Y	Y	8459	47686	10	1	5	5	25	1	5	3	15	50
Ivisan	15	Y	Y	Y	Y	5223	28702	6	1	5	3	15	1	5	1	5	30
Jamindan	30	Y	Y	Y	Ν	6683	40186	8	1	5	6	30	1	5	0	0	40
Maayon	32	Y	Y	Y	Ν	7411	38687	8	1	5	5	25	2	10	0	0	40
Mambusao	26	Y	Y	Y	Y	8220	43533	9	1	5	4	20	1	5	3	15	45
Panay	42	Y	Y	N	Y	9162	48036	10	1	5	6	30	0	0	3	15	50
Panitan	26	Y	Y	Y	Y	8033	44320	9	1	5	5	25	0	0	3	15	45
Pilar	24	Y	Y	Y	Y	8165	46031	9	1	5	4	20	1	5	3	15	45
Pontevedra	26	Y	Y	Y	Y	9141	47449	10	1	5	5	25	1	5	3	15	50
President Roxas	22	Y	Y	Y	Y	5842	32573	8	1	5	4	15	1	5	2	10	35
Sapian	10	Y	Y	N	Ν	5105	27109	5	1	5	4	20	0	0	0	0	25
Sigma	21	Y	Y	Y	Y	6260	32380	9	1	5	5	15	1	5	2	10	35
Tapaz	58	Y	Y	Y	Y	9384	52164	10	1	5	5	25	1	5	3	15	50
Roxas City	47	Y	Ν	Ν	Y	27817	148809	30	5	20	15	70	0	0	10	50	140
TOTAL	473	17	16	13	13	142305	773300	160	21	100	89	420	12	60	38	190	770

Table 3: Research and Sampling Design

3.2: Sample Collection

Samples were collected from the source directly or from the point of use by the author or Municipal Sanitary Inspector. In some circumstances where the point of use location was not sufficient for sampling (e.g. a storage tank that was no longer filled with water), then samples were collected from the household storage containers. To ensure sterile conditions, water samples were collected into two separate containers: sterile 100-mL polystyrene vessels for laboratory testing and 100-mL sterile sampling bags for EC-Kit field testing.

The following diagram (Figure 6) summarizes the general water sampling and testing methodology used by Provincial Health Office and the author in the field research.

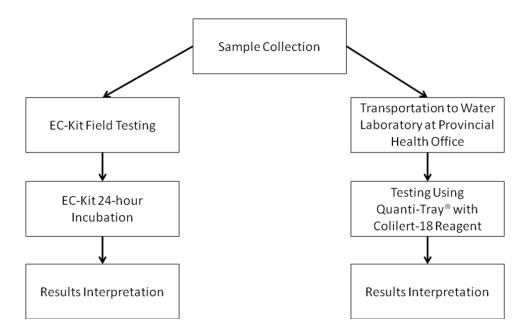


Figure 6: Flow diagram representing sampling/testing methodology.

The water samples were identified utilizing a labeling system created by members of the Capiz Provincial Health Office. The labels included abbreviations per municipality (Table 4), *barangay* number, water source level and type of water source (Table 5), date of sampling, and time of sampling. Furthermore, the samples were also given unique numbers per daily sampling batch. An example of a labeled water sample is shown in Figure 7, which was collected from a

deep well pump (a Level 2 water source) in *barangay* Tapaz, at 11:03 AM on January 20, 2010. It was also the fifth collected sample of the day.

Municipality Name	Abbreviation
CUARTERO	CU
DAO	DA
DUMARO	DR
DUMALAG	DU
TAPAZ	ТА
MAAYON	MA
PILAR	PI
PONTEVEDRA	PV
PRESIDENT ROXAS	PR
SIGMA	SG
MAMBUSAO	MB
SAPIAN	SP
JAMINDAN	JM
PANAY	PY
PANITAN	PT
IVISAN	IV
ROXAS CITY	RX

Table 4: Municipality Codes

Water Source Level	Water Source Code	Water Source Descriptions
Doubtful (D)	OD	Open dug well
	US	Unprotected spring
	SW	Surface water (Rivers, streams, creeks)
	ОТ	Others not mentioned above
	SWP	Shallow well with pump (<60 ft)
	JMP	Jetmatic Pump with or without motor
Level 1	DWP	Deep well with pump (>60 ft)
(L1)	PDW	Protected dug well
	PS	Protected dug well without distribution
	RW	Rain water catchments (ferro cement tanks)
Level 2	GPS	Gravity protected spring with pipe distribution, Communal tap stands
(L2)	DWP	Deep well with pump with pipe distribution, Communal tap stands
Level 3 (L3)	WD	Water Districts
	LWUA	Local water utilities administration
	BAWASA	Barangay waterworks system

Table 5: Water Source Codes and Descriptions

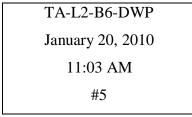


Figure 7: Example of a Labeled Water Sample

CHAPTER 4: METHODOLOGY

4.1 Procedure for Quanti-Tray® Test

The Quanti-Tray® test was utilized as the standard against which the EC-Kit was compared (see MIT teammate thesis of Trottier, 2010 for hydrogen sulfide bacteria and Easygel® field test kit comparisons with Quanti-Tray®). After collection of 100-mL water samples in the sterile 120-mL capacity vessels³, the samples were placed in ice chests containing ice or ice packs and taken to the water laboratory at the Capiz Provincial Health Office. The Provincial Health Office and MIT team used the following procedure to run the Quanti-Tray® test:

Quanti-Tray® Test Procedure⁴:

1. Open snap pack⁵ and add the reagent to 100 mL of water sample in a sterile 120-mL vessel.



³ IDEXX WV120SBST-200: 120-mL Shrink-banded vessels with sodium thiosulfate.

⁴ Quanti-Tray procedure photos from IDEXX website (IDEXX, 2009).

⁵IDEXX WP200I: Colilert®-18 Snap packs for 100-mL sample, as used in Capiz Province, Philippines; IDEXX WP200I: Colilert® Snap packs for 100-mL sample, as used in Cambridge, Massachusetts.

 Use one hand to hold a Quanti-Tray®⁶ upright with the well side facing the palm. Squeeze the upper part of the Quanti-Tray® so that the Quanti-Tray® bends toward the palm.



- 3. Gently pull foil tab to separate the foil from the tray. Avoid touching the inside of the foil or tray.
- 4. Pour the reagent/sample mixture directly into the Quanti-Tray®, avoiding contact with the foil tab.



- 5. Tap the small wells 2–3 times to release any air bubbles. Allow foam to settle.
- 6. Place the sample-filled Quanti-Tray® onto the rubber insert of the Quanti-Tray® Sealer with the well side (plastic) of the Quanti-Tray® facing down.



- 7. Seal according to the Quanti-Tray® Sealer instructions.
- Incubate according to reagent instructions (at least 18 hours for Colilert®-18 reagent, and at least 24 hours for Colilert® reagent).

⁶ IDEXX WQT100: Quanti-Tray®, as used in Capiz Province, Philippines; IDEXX WQT-2K: Quanti-Tray®/2000, as used in Cambridge, Massachusetts.

Interpreting Results:

After incubation, a positive sample for total coliform turns yellow and a negative sample looks the same visually as when it was collected. A positive sample for *E.coli* fluoresces blue under ultraviolet (UV) light.⁷ The Most Probable Number (MPN) is obtained by counting the positive wells and using the appropriate Quanti-Tray® table to find the MPN (see Appendix A: IDEXX 51-Well Quanti-Tray® MPN Table).

4.2 Procedure for EC-Kit: Colilert[®] and PetrifilmTM

The EC-Kit instructions included in each EC-Kit describe the steps to perform two complementary indicator tests, 10-mL predispensed Colilert®⁸ tubes and PetrifilmTM. The instructions also include setup and quality control and interpretation procedures. After collecting water samples in the sterile sampling bags, the samples were placed in ice chests containing ice or ice packs and taken to the municipal health offices for processing by the Municipal Sanitary Inspector or the author and recorded (see Appendix B: Water Quality Results, Sorted by Municipality).

⁷ Chauvet NV-F4 Handheld Blacklight With Flashlight. Supplier: Musician's Friend. <u>http://pro-audio.musiciansfriend.com/product/Chauvet-NVF4-Handheld-Blacklight-With-Flashlight-?sku=800098</u>

⁸ Colilert® 10-mL predispensed test reagent is the identical reagent to that used in the Quanti-Tray® test. However, the difference is in the sample size (10-mL vs. 100-mL) and therefore in the detection limits. The detection limit of the Colilert(R) 10-mL tubes is 10 cfu per 100 mL of sample (a positive/present Colilert(R) result indicates 1 cfu per 10 mL sample, and hence 10 cfu per 100 mL of sample). The detection limit of Quanti-Tray® is 200.5 MPN per 100 mL of sample, whereas the detection limit of Quanti-Tray®/2000 is 2419 MPN per 100 mL of sample.

4.2.1 Procedure for Colilert® (10-mL Predispensed Tubes)

Colilert® (10-mL Predispensed Tubes) Preparation:

- 1. Wash hands with soap and water.
- 2. Locate a clean, level surface. Cover surface with a large plastic garbage bag, taped down with masking tape. Or, use a square ceramic or plastic tile as a work surface. Wipe down work surface with isopropyl (rubbing alcohol).
- 3. Using the black-marked 10 milliliter (mL) guide test tube provided (the one tube with colored tape in the package), mark <u>all</u> the other test tubes in the kit with a permanent black marker at the same 10 mL level.
- 4. Label each tube with the appropriate sample name, time, date of sample collection, and initial of person sampling. Ensure that all test results are recorded in a lab notebook.

Colilert® (10-mL Predispensed Tubes) Test Procedure

- 1. Remove cap, without touching the inside of the cap with fingers or hand.
- 2. Then fill the Colilert® test tube with 10mL of sample water to that level by pouring directly from bag into the tube, or use the sterile pipette provided in kit (graduated at 1 mL) to transfer sample water from the plastic bag to the test tube 10 times. Take care not to touch the sides of the tube or the water in the tube with the pipette.
- 3. Replace the cap and mix the water in the test tube by inverting it several times to dissolve the nutrients.
- 4. Put Colilert® tube in top pocket of incubator belt, tie the incubator belt around your waist and wear it nonstop for 24 hours +/- 2 hrs to incubate the water sample using body heat.
- 5. Run blanks and duplicates minimum of 5% of total samples tested using boiled, cooled water, or bottled water, to assure quality.

Interpreting Colilert® Results:

After 24 hours, if samples are clear, or visually the same as when collected, then no coliform bacteria are present (see top tube in Figure 8). If samples are slightly yellow or yellow, coliform bacteria are present (see middle and bottom tubes displayed in Figure 8). Record this as clear (absent) and yellow (present) on data sheets. If the samples fluoresce to form a milky-blue color under UV/black light, then *E. coli* are present (see bottom tube in Figure 9). Otherwise, if the sample does not fluoresce, then *E.coli* are not present (see top two tubes in Figure 9). NOTE: Two tubes in Figure 9 show UV/black light reflecting off the Colilert® tube glass. This is not fluorescence! If *E.coli* are present, a PetrifilmTM test should also be performed in order to quantify (if sample risk is unknown, perform both tests).



Figure 8: Coliform Results of 10-mL Colilert® Tube Test

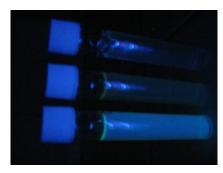


Figure 9: E.coli Results of 10-mL Colilert® Tube Test

4.2.2 Procedure for PetrifilmTM Test

The PetrifilmTM test uses sample-ready plates to quantify the level of *E. coli* and total coliform. The PetrifilmTM test quantifies *E.coli* and total coliforms with a minimum detection limit of 1 *E.coli* per 1 mL (high risk). The pre-coated PetrifilmTM contains: Violet Red Bile (VRB) nutrients (a gelling agent), BCIG (5-bromo-4-chloro-3-indolyl β-D-glucuronide, an indicator of glucuronidase activity), tetrazolium (indicator that enables the developed colonies to be visually counted), and a top film on the plate that traps gas produced by lactose fermenting *E. coli* and coliforms.

Petrifilm Procedure:

- 1. Place the PetrifilmTM on a flat surface that has been wiped down with isopropyl/rubbing alcohol.
- Fill sterile pipette with 1 mL of sample water (1 mL= top graduated line just below top of pipette bulb)
- Lift the top film. With pipette perpendicular to PetrifilmTM plate, carefully dispense the 1 mL of sample from the pipette on to the center of the pink circle.
- 4. Gently roll the top film onto the PetrifilmTM plate. Take care not to trap air bubbles under the top film.
- 5. Allow the water to naturally spread out to fill the entire pink circle.
- 6. Place the PetrifilmTM between two pieces of cardboard. Secure the PetrifilmTM between the cardboard using rubber bands.
- Place PetrifilmTM samples in bottom pocket of incubator belt. Up to five Petrifilms can be stacked between one set of cardboard squares. Incubate at body temperature for 24 hours +/- 2 hours.

Interpreting PetrifilmTM results:

E.coli are blue colonies with gas bubbles. Total coliform results are the sum of red colonies with gas bubbles plus blue colonies with gas bubbles. It is highly recommended that the PetrifilmTM *E.coli*/Coliform Count Plate Interpretation Guide be utilized in analysis of PetrifilmTM results⁹.

Bubble patterns may vary. Gas may disrupt the colony so that the colony "outlines" the bubble (circles 1 and 2 in Figure 10). Artifact bubbles may result from improper inoculation or from trapped air within the sample. They are irregularly shaped and are not associated with a colony (circle 3 in Figure 10). Figure 11 shows various bubble patterns associated with gas producing colonies. All should be enumerated.

If the total number of blue colonies with gas bubbles is less than 1, then the water may still have an intermediate risk level that is below the detection limit of the PetrifilmTM test. If the total number of blue colonies with gas bubbles counted is between 1 and 10, this represents a high risk level. If the total number of blue colonies with gas bubbles counted is above 10, this is a very high risk level.

⁹ Document available for download under "Instructions for Use" at the 3M website: <u>http://solutions.3m.com/wps/portal/3M/en_US/Microbiology/FoodSafety/product-information/product-catalog/?PC_7_RJH9U523003DC023S7P92O3O87_nid=C0WJ62882Vbe29BDXSBJ7Fgl</u>

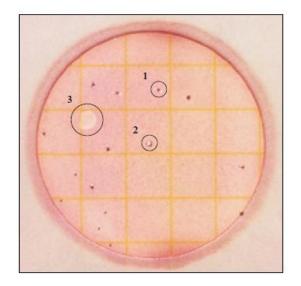


Figure 10: Sample Total Coliform Count Plate (3M, 2001). Circles 1 and 2 are associated with colonies with gas bubbles, circle 3 is not associated with a colony.

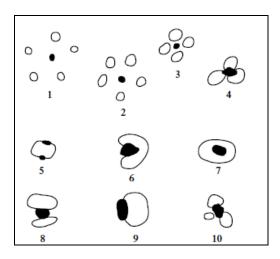


Figure 11: Various Bubble Patterns for Gas Producing Colonies (3M, 2001).

4.2.3 Recommendations on Reading Colilert® and PetrifilmTM Results

For the Colilert[®] test, the UV/black light test to determine fluorescence must be performed in the dark (dark room, a closet, a bathroom, or outdoors at night). Otherwise, fluorescence will not be able to be seen clearly.

For the PetrifilmTM test, it must be read in bright daylight (hold the PetrifilmTM up to natural light). Furthermore, it must be counted systematically by using the grid system on the PetrifilmTM plate (see Figure 12). Begin at the top right square and proceed sequentially from square to square following the curved "S" path on the figure below. Colonies on the horizontal grid lines are "pushed down into the square below." Colonies on the vertical grid lines are pulled forward into the next square (see Figure 12). It is vital to count every colony: blue with gas bubbles, red with gas bubbles, then add blue with gas bubbles and red with gas bubbles (including even very small colonies with gas bubbles).

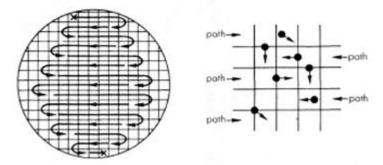


Figure 12: System for Counting Coliform Colonies on PetrifilmTM

4.2.4 Determining Risk Levels from EC-Kit Results

For the purposes of prioritizing interventions at the regional level, it is important to monitor the improvement or deterioration of drinking water supplies. In many developing countries, a high proportion of small-community drinking-water systems fail to meet requirements for water safety (World Health Organization, 2006). According to the WHO, assigning a grading scheme is particularly useful for water sources where water quality testing frequency is low. Furthermore, in locations where community water supplies are unchlorinated, as was the case for non-Level 3 water sources in Capiz Province, they will inevitably contain large numbers of total coliform bacteria. Therefore, it was recommended that the bacteriological classification scheme should be based on thermotolerant (fecal) coliform bacteria or *E.coli* (World Health Organization, 1997). For this study, analysis of microbiological water quality data was divided into the WHO risk levels shown in Table 6.

Table 6: Classification and Color-code Scheme for Thermotolerant (fecal) Coliforms or *E.coli* inWater Supplies (World Health Organization, 1997)

Count per 100 ml	Category and colour code	Remarks
0	A (blue)	In conformity with WHO guidelines
1-10	B (green)	Low risk
10-100	C (yellow)	Intermediate risk
100-1000	D (orange)	High risk
>1000	E (red)	Very high risk

The results of the two indicator tests that comprise the EC-Kit enable the determination of different risk levels. The two right-hand columns of Table 7 show the aforementioned World Health Organization's risk rankings for *E.coli* (World Health Organization, 1997). At less than 10 *E.coli* colony forming units (cfu) per 100 mL sample, WHO quantifies risk of waterborne disease as low. Looking at the "Colilert®" column, low risk is a negative result. A water sample with at least one *E.coli* per 10 mL Colilert® test (a result that comes out positive) and no presence of *E.coli* on the PetrifilmTM, shows intermediate risk between 10 – 100 colony counts per 100 mL. High and Very High risk waters are identified by positive Colilert® results and 1-10 (High) or >10 (Very High) *E.coli* counts on PetrifilmTM test.

EC-Kit Resu (Metcalf, 200		Risk Level Categories (World Health Organization, 1997)		
Colilert® <i>E. coli</i> Result	Petrifilm TM E. coli Result	Risk Level	<i>E.coli</i> in sample (cfu/100 mL)	
Absent (no fluorescence)	0	Conformity	< 1	
Absent (no fluorescence)	0	Low	1-10	
Present (blue fluorescence)	0	Intermediate	10-100	
Present (blue fluorescence)	1-10 (blue with gas bubbles count)	High	100-1000	
Present (blue fluorescence)	> 10 (blue with gas bubbles count)	Very High	> 1000	

Table 7: Determining Risk Level Using EC-Kit Results

4.3 Charles River Water Sampling

On April 4, 2010, the author collected water samples from the Charles River at the MIT Sailing Pavilion, in Cambridge, Massachusetts. The samples were obtained in one location, versus multiple locations (as was the case in Capiz Province). The purpose of the study was to conduct a detailed, in-depth controlled experiment comparing the EC-Kit field test with the Quanti-Tray® Standard Method with multiple sample dilutions, duplicates, and blanks. A secondary purpose was to refine the comparison by running tests using Quanti-Tray®/2000 versus Quanti-Tray® (see Appendix C: Charles River Water Quality Results).

Charles River Water Sample Collection Procedure:

- 1. Rinse out sampling containers with Charles River water (the sampling vessels used in this study were standard five-gallon plastic containers).
- 2. Collect sufficient Charles River Water (this study collected 10 gallons, which was more than enough) and carry to the laboratory for testing.
- 3. Set up and label Quanti-Tray® and EC-Kit tests with date, dilution, and sample number.
- 4. Stir the Charles River water samples.
- Dilute samples in sterile beakers with the appropriate volumes of deionized water. For example, a dilution of 1/100 would have 1 mL of Charles River Water in 100 mL of deionized of water.
 - a. Rinse beakers with deionized water in between each dilution.
 - b. Note: The dilutions should be conducted in increasing magnitude (e.g. 1/100 first, followed by 2/100).
- 6. Conduct Quanti-Tray® and EC-Kit tests according to procedures detailed in Section 4.1 and 4.2.
- 7. Incubate for 24 hours using laboratory incubator.

4.4 GPS Testing

For the drinking water quality mapping aspect of the study, the author collected the coordinates for 160 water samples in January 2010 using a GARMIN® eTrex® Handheld Global Positioning System (GPS) (Figure 13). While there are 521 water quality samples from Capiz Province, the author was only able to collect the coordinates for 160 sources because many of the samples were collected after the month of January. The GPS coordinates were collected at each water source and sampled by the author. The Quanti-Tray® and EC-Kit water quality results were recorded for each of the 160 sources and entered into the map (see Appendix D: GPS Coordinates).



Figure 13: Garmin® eTrex® GPS

4.5 Statistical Analyses

The Capiz Province and Charles River water samples were analyzed statistically to determine the accuracy of the water quality tests compared to the Standard Method, IDEXX Quanti-Tray®. For all statistical analyses, STATA: Data Analysis and Statistical Software (Version 11.0) was used.

One of the main difficulties encountered in the comparisons of Colilert®, PetrifilmTM, and the EC-Kit as a whole to Quanti-Tray® and Quanti-Tray®/2000 is the varying detection limits per test. While the Colilert® 10-mL predispensed test reagent is identical to that used in the Quanti-Tray® test, the difference is in the sample size (10-mL vs. 100-mL) and therefore in the detection limits. The detection limit of the Colilert® 10-mL tubes is 10 cfu per 100 mL of sample (a positive/present Colilert® result indicates 1 cfu per 10 mL sample, and hence 10 cfu per 100 mL of sample). The detection limit of Quanti-Tray® is 200.5 MPN per 100 mL of sample, whereas the detection limit of Quanti-Tray®/2000 is 2419 MPN per 100 mL of sample.

Another difficulty is the fact that the Colilert® test is a qualitative test and the PetrifilmTM test is enumerative. Statistical analyses of the two microbiological tests were conducted through the use frequency distribution tables, otherwise known as contingency tables. Contingency tables represent a method for analyzing categorical/nominal data (e.g. Presence/Absence, or WHO risk levels) and are a simple procedure for reviewing how different categories are distributed in the samples. The frequency distribution tables were then tested for statistical significance through the chi-square test or Fisher's exact test.

Statistical significance reports if it is unlikely a result occurred by chance, and establishes the degree of confidence that one can have in making a inference from a sample to its parent population (Meier, Brudney, & Bohte, 2009). The level of statistical significance is determined through the use of a p-value (Rosner, 2006) as shown in Table 8.

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

Table 8: Guidelines for assessing the significance of a *p*-value (Rosner, 2006).

The chi-square test compares the frequencies actually observed with the expected frequencies (presuming no relationship between the variables) throughout the contingency table. 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 (χ 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).

Since the chi-square test can only be computed for a 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 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).

4.6 Limitations to the Study

One of the challenges in analyzing the Quanti-Tray and EC-Kit data for the Capiz Province water samples was that the detection limits of the Quanti-Tray® used in the Philippines is 200.5 MPN/100 mL of sample, whereas the detection limit of Quanti-Tray®/2000 used for Charles River water samples is 2419 MPN/100 mL of sample.

The Quanti-Tray[®] was likely recommended to the Capiz Provincial Health Office by the supplier/distributer because Capiz indicated they were testing drinking water samples. However, the supplier/distributer may have been unaware of the potential that the *E.coli* contaminant levels for the drinking water samples might fall in the range between 200 and >2000 cfu, or perhaps even higher.

Meanwhile, the PetrifilmTM has a higher detection limit. As seen in Table 7, detection of one colony forming unit (cfu) on the PetrifilmTM already places the water sample in the High or Very High risk level category. The PetrifilmTM's inability to distinguish between Conformity, Low, and Intermediate is one of the primary reasons for needing the additional 10-mL predispensed Colilert® test in the EC-Kit.

CHAPTER 5: EC-KIT COMPARISON TO QUANTI-TRAY®

This chapter presents the results of the EC-Kit verification part of this overall study. It provides the water quality results collected by the author for 521 water samples collected in Capiz Province as well as the water quality results for 40 water samples from the Charles River in Massachusetts. Each sample was tested in the field using the two component tests of the EC-Kit (Colilert® and PetrifilmTM) and in the laboratory using the enzyme substrate method (Quanti-Tray®). All samples were concurrently tested for *E. coli* and total coliform. Note however, that all results in this chapter report on *E.coli* contamination only, not total coliform.

The Colilert® and PetrifilmTM test results are compared to the Quanti-Tray® test results for Capiz Province first, followed by a similar comparison for Charles River samples. Then, the results of the EC-Kit in Capiz Province are compared to the Quanti-Tray®. The purpose of comparing these tests to Quanti-Tray® is to verify the accuracy of each test to the standard, where accuracy reflects the closeness of the measured value (EC-Kit) to a true value (Quanti-Tray®). Likewise, the comparison for Charles River water samples is to verify each test to the standard, with the distinction of using Quanti-Tray®/2000 instead of Quanti-Tray®.

5.1 Colilert®/Quanti-Tray® Comparison and PetrifilmTM/Quanti-Tray® Comparison for Capiz Province Water Samples

From Table 9, Colilert® and Quanti-Tray® complied with one another for 388 (242+146) of the 521 samples, and 133 (101+32) samples that did not comply with each other. Of the 133 samples, 32 of these were false positives and 101 were false negatives.

From Table 10 it can be seen that PetrifilmTM and Quanti-Tray® yielded the same result 459 (353+106) out of the 521 samples. And there were 62 (43+19) out of 521 samples where the two tests were not congruent with each other. Of these 62 samples, 19 were false positives and 43 were false negatives. The false negatives had PetrifilmTM indicating a higher risk level category than Quanti-Tray®. While ideally one would hope for perfect congruence of the two test methods, it is preferable to have over-reporting of risk (false positives) than under-reporting of risk (false negatives).

A Pearson's chi-square test was conducted on the Capiz Province test results. The chisquared ($\chi 2$) values for both Table 9 and Table 10 were 129.923 and 254.3837, respectively, both of which resulted in Pr = 0.000, which demonstrates that the correlation between each Colilert® and PetrifilmTM with Quanti-Tray® is statistically significant.

 Table 9: Frequency Distribution Table of Colilert® and Quanti-Tray® for *E.coli* Contamination

 for Capiz Province

		Quanti-Tray®		
		Presence	Absence	Total
Colilert®	Presence	242	32	274
	Absence	101	146	247
	Total	343	178	521

Table 10: Frequency Distribution Table of PetrifilmTM and Quanti-Tray® for *E.coli* Contamination for Capiz Province

	Quanti-Tray® Most Probable Number			
	Risk Level	Conformity/Low/Intermediate	High/Very High	Total
Petrifilm TM	Conformity/Low/Intermediate	353	19	372
High/Very High		43	106	149
	Total	396	125	521

5.2 Colilert®/Quanti-Tray® Comparison and PetrifilmTM/Quanti-Tray® Comparison for Charles River Water Samples

In addition to the data for water samples in the Philippines, the author conducted a study on dilutions of Charles River water. The data from the Charles River water were likewise analyzed through the use of frequency distribution tables. As previously discussed in Section 4.6 Limitations to the Study, the primary difference between the water quality results for Capiz and for Charles River is the detection limits for Quanti-Tray® compared to Quanti-Tray®/2000. The use of Quanti-Tray®/2000 for Charles River water allowed for distinction between the High and Very High risk levels because of its higher detection limit of 2419 MPN/100 mL of sample versus 200.5 MPN. Furthermore, the author wanted to investigate the repeatability of Quanti-Tray® results for identical samples (which was not done in The Philippines), so dilution samples were conducted in duplicates in addition to two blanks (Table 12).

 Table 11: Frequency Distribution Table of Colilert® and Quanti-Tray®/2000 for E.coli

 Contamination of Charles River Water

		Quanti-Tr		
		Presence	Absence	Total
Calilardo	Presence	38	0	38
Colilert®	Absence	0	2	2
	Total	38	2	40

 Table 12: Frequency Distribution Table of PetrifilmTM and Quanti-Tray® for *E.coli*

 Contamination of Charles River Water

		Quanti-Tray®/2000 Most Probable Number				
	Risk Level	Conformity/Low	Intermediate	High	Very High	Total
Petrifilm TM	Conformity/Low/Intermediate	4	10	5	0	19
retriinii	High/Very High	0	0	17	4	21
	Total	4	10	22	4	40

Due to the smaller sample size, a Fisher's exact test instead of Pearson's chi-square test was conducted on the Charles River water. The Fisher's exact test for Table 11 and Table 12 yielded a value of 0.001 and 0.000, respectively, which demonstrates that the correlation between each Colilert® and PetrifilmTM with Quanti-Tray® is statistically significant.

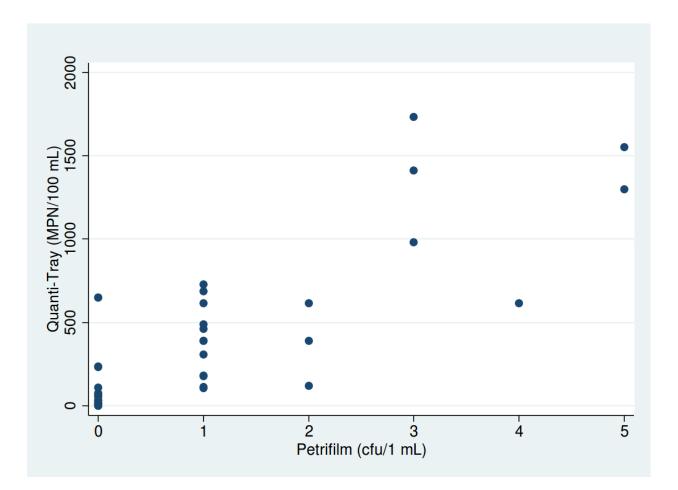


Figure 14: Quanti-Tray® and PetrifilmTM Water Quality Results for *E.coli* for Capiz Province and Charles River Water for Different Risk Levels

5.3 EC-Kit (Combined Colilert® and PetrifilmTM) and Quanti-Tray® Comparison Results for Both Capiz Province and Charles River Water

Utilizing the information gathered from both the Colilert® and PetrifilmTM tests, it is possible to create a 3x3 frequency distribution table comparing EC-Kit results with Quanti-Tray® (Table 13 and Table 14).

 Table 13: Frequency Distribution Table of EC-Kit and Quanti-Tray® for *E.coli* Contamination of Capiz Province

		Quanti-Tray	Quanti-Tray® Most Probable Number			
	Risk Level	Conformity/Low	Intermediate	High/Very High	Total	
	Conformity/Low	230	13	4	247	
EC-Kit	Intermediate	76	34	15	125	
	High/Very High	13	30	106	149	
	Total	319	77	125	521	

Table 14: 3x3 Frequency Distribution Table of EC-Kit and Quanti-Tray® for *E.coli*Contamination of Charles River Water

		Quanti-Tray	Quanti-Tray® Most Probable Number			
	Risk Level	Conformity/Low	Intermediate	High/Very High	Total	
	Conformity/Low	2	0	0	2	
EC-Kit	Intermediate	2	10	5	17	
	High/Very High	0	0	21	21	
	Total	4	10	26	40	

For graphical representation, the Quanti-Tray® Most Probable Number (MPN) were slightly altered: MPN values <1 were changed to 0, and any MPN values >200.5 were changed to 201. Furthermore, values of "Too Numerous to Count (TNTC)" for PetrifilmTM was changed to 20 cfu /1 mL. Figure 15 shows the scatter plot with the aforementioned values adjusted, and is

essentially a graphical representation of Table 10. The green shaded quadrants represent samples in which the PetrifilmTM results were congruent with the Quanti-Tray® results. Moreover, while Figure 15 shows all samples (N=521), there are not 521 data points shown because many of the data points had the same PetrifilmTM and Quanti-Tray® values and so one point may represent multiple occurrences.

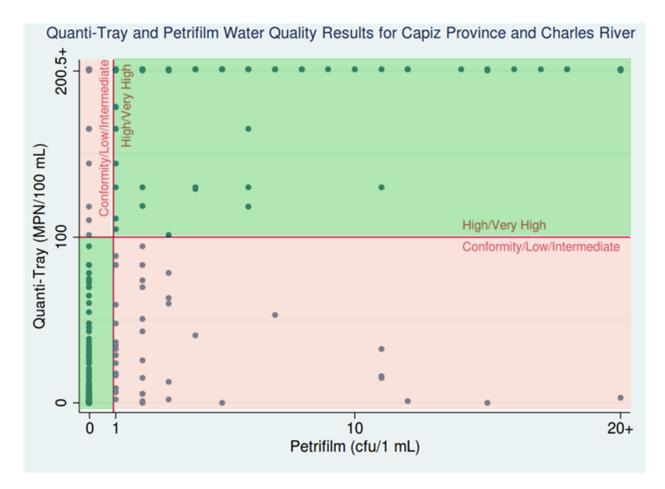


Figure 15: Quanti-Tray® and PetrifilmTM Water Quality Results for Capiz Province and Charles River

CHAPTER 6: ACCURACY OF EC-KIT IN REDUCING ERROR RELATIVE TO IMPROVED/UNIMPROVED WATER SOURCE DESIGNATION

In assessing the accuracy of the EC-Kit and its components as a means of testing water quality, there were two statistical analyses used. Given the designations of unimproved and improved water sources, the author assumed that an unimproved water source corresponded to the Philippines designation of a doubtful water source, and an improved water source corresponded to levels 1 through 3.

To calculate error and reduction in error, we used the following formula:

$$\lambda = \frac{(\text{Error without conditional information}) - (\text{Error with conditional information})}{(\text{Error without conditional information})}$$

This formula, as applied to the statistical analyses of this study, may be rewritten as:

$$\lambda = \frac{(\text{Error from knowing water source type}) - (\text{Error from knowing water source type and additional water quality test})}{(\text{Error from knowing water source type})}$$

The λ value, defined as "proportional reduction in error," is a measure of how good one becomes at making predictions. In other words, λ , the proportional reduction in error, represents how much knowledge one gains by obtaining water quality test results compared to assuming contamination level based on improved/unimproved water source type. For example, given an unimproved water source, one would assume that the water source was contaminated. This assumption, based on the water quality results from Capiz using the Quanti-Tray test, would be incorrect 15% of the time. The results of calculation error and λ are in Table 15 for the various options.

Tests	Error	Proportional Reduction in Error (λ)
Unimproved + Quanti-Tray®	15%	
Unimproved + Colilert®	12%	25%
Unimproved + Petrifilm TM	37%	-138%
Unimproved + EC-Kit (exact match)	25%	-63%
Unimproved + EC-Kit	6%	63%
Improved + Quanti-Tray®	64%	
Improved + Colilert®	27%	58%
Improved + Petrifilm TM	39%	39%
Improved + EC-Kit (exact match)	29%	54%
Improved + EC-Kit	6%	60%

Table 15: Error and Proportional Reduction in Error for Unimproved and Improved Water Sources

The error for improved and unimproved water sources with Colilert® and PetrifilmTM tests reported the percentage frequency of the red-shaded areas in Table 16. For unimproved water sources, the addition of only a PetrifilmTM would actually lead the user to err more often, whereas the addition of only a Colilert® test would improve predictions by 25% (and hence resulting in a 12% error instead of 15%). For improved water sources, the addition of a Colilert® or PetrifilmTM test would improve guesses by 58% and 39% respectively.

Table 16: Calculations for Error for Colilert® or PetrifilmTM

		Quanti	-Tray®
			Absence
Water Source Type	Presence		
+ Additional Test	Absence		

Table 17: Calculations for Error for EC-Kit (exact match)

		Q	uanti-Tray®	
		Conformity/Low	Intermediate	High/Very High
	Conformity/Low			
Water Source Type + EC-Kit	Intermediate			
T EC-NI	High/Very High			

Table 18: Calculations for Error for EC-Kit

		Quanti-Tray®		
		Conformity/Low	Intermediate	High/Very High
Water Source Type + EC-Kit	Conformity/Low			
	Intermediate			
	High/Very High			

The calculations for error for EC-Kit were done in two ways. The "exact match" method reports the percentage frequency of the red-shaded areas in Table 16 and

Table 17. This is useful information for verification of EC-Kit, but not as useful for water quality testing, meaning that with the EC-Kit, an overestimate of the risk level still yields useful information for someone interested in testing their water quality. So, the error values of interest actually follow the red-shaded areas in Table 18.

Therefore, for both unimproved and improved water sources, the addition of both tests in the form of an EC-Kit would improve predictions by 60-63% (highlighted rows in yellow in Table 15).

CHAPTER 7: DRINKING WATER QUALITY AND RISK LEVELS

Of the 521 drinking water samples collected in Capiz Province, 10% were unimproved (doubtful) and 90% were improved (levels 1, 2, and 3) (Table 19). This is very close to the distribution of 8% unimproved and 92% improved water sources shown in Table 2, and thus our sample is fairly representative of the water sources in Capiz Province.

Water Source Level	Number of Samples	Percent of Total Samples Collected		
Doubtful	52	10%		
Level 1	339	65%		
Level 2	62	12%		
Level 3	68	13%		
Total	521	100%		

Table 19: Distribution of the 521 Drinking Water Samples Collected in Capiz Province

A summary of the *E.coli* levels, and therefore WHO risk levels, for the 521 drinking water samples collected in Capiz Province are presented in Table 20 and Table 21. According to Table 20, the mean MPN *E.coli* was about 53 cfu per 100 mL, which corresponds to a WHO Intermediate risk level. Even this number is a conservative estimate, because the author assumed that all ">200.5 MPN" values were equal to 201 for calculation purposes. To delve further, the average of all samples, excluding the ">200.5 MPN" values still fell within the Intermediate risk level with a mean MPN of 20 *E.coli* cfu per 100 mL. The mean *E.coli* count was about 4 colonies, which corresponds to a High risk level (Table 21). For calculation purposes, the TNTC values were set to 100 colonies, but even with the exclusion of such values, the mean PetrifilmTM *E.coli* counts still fell within the High risk level.

Test	Number of Samples	Mean MPN <i>E.coli</i>	Standard Deviation	Minimum Value	Maximum Value
Quanti-Tray® MPN <i>E.coli</i>	521	52.80672	80.62943	0	201
Quanti-Tray® MPN <i>E.coli</i> (Excluding >200.5 values)	426	19.7589	44.16539	0	200.5

Table 20: Mean Quanti-Tray® MPN E.coli Levels for Capiz Province

Table 21: Mean PetrifilmTM E. coli Counts for Capiz Province

Test	Number of Samples	Mean <i>E.coli</i> count	Standard Deviation	Minimum Value	Maximum Value
Petrifilm TM <i>E.coli</i> Counts	521	3.96161	14.92682	0	100
Petrifilm TM <i>E.coli</i> Counts (Excluding TNTC)	511	2.08219	6.541354	0	63

A classification of the Quanti-Tray® and PetrifilmTM results per water source level are shown in Table 22 and Table 23 (with >200.5 MPN and TNTC values included). As expected, the mean *E.coli* values decrease as the water sources improve (e.g. moving from doubtful, level 1, level 2, level 3).

Since the >200.5 MPN values were converted to 201 MPN, the Quanti-Tray® results in Table 22 are conservative. According to the Quanti-Tray® mean MPN *E.coli* results, the doubtful sources fall within the High risk level, the level 1 and level 2 sources fall within the intermediate risk level category, and the level 3 sources fall within the low risk level category. In other words, on average, none of the collected water samples collected in Capiz Province conforms to the WHO guidelines for safe drinking water.

According to the PetrifilmTM mean *E.coli* counts (Table 23), the doubtful, level 1, and level 2 sources all fall within the High risk level, and the level 3 sources fall within the conformity/low/intermediate risk level category (note that even with the exclusion of the TNTC)

value of 100, the risk levels remain unchanged). The distinction between the conformity/low/intermediate risk level categories relies on the Colilert® 10-mL predispensed tube results. Of the 68 level 3 sources, 15 had a positive (present) Colilert® result, and fell within the intermediate risk level category and 53 had a negative (absent) Colilert® result, and fell within the conformity/low risk level category.

Water Source Level	Number of Samples	Mean MPN <i>E.coli</i>	Standard Deviation	Minimum Value	Maximum Value
Doubtful	52	102.828	95.19513	0	201
Level 1	339	60.4	84.96928	0	201
Level 2	62	18.7645	38.05484	0	201
Level 3	68	7.73824	21.2141	0	201

Table 22: Mean Quanti-Tray® MPN E.coli per Water Source Level for Capiz Province

Table 23: Mean PetrifilmTM E.coli Count per Water Source Level for Capiz Province

Water Source Level	Number of Samples	Mean <i>E.coli</i> count	Standard Deviation	Minimum Value	Maximum Value
Doubtful	52	6.01923*	16.4239	0	100
Level 1	339	5.059**	17.1569	0	100
Level 2	62	0.5	1.80845	0	12
Level 3	68	0.07353	0.43421	0	3

*When excluding the TNTC value of 100, the Mean *E.coli* count was 4.17647. **When excluding the TNTC value of 100, the Mean *E.coli* count was 2.4697.

CHAPTER 8: DRINKING WATER QUALITY MAPPING

This chapter discusses the map of EC-Kit and Quanti-Tray® risk level results. Using a GARMIN® eTrex® Summit Global Positioning System (GPS) the author recorded the locations of 160 collected water sources and analyzed the samples for *E.coli* and total coliform. To ensure accuracy, the coordinates were collected simultaneously with the water samples.

With the expert guidance and technical support efforts of Jonathan Lowe at GISwebsite, the author created a water map of the drinking water quality risk results for these 160 data points. Currently, the website displays each data point as a bubble, color-coded according to WHO risk level results (Figure 16). Users are able to view and sort the risk level results from both Quanti-Tray® and EC-Kit test procedures by water source type, municipality, and *barangay* name.

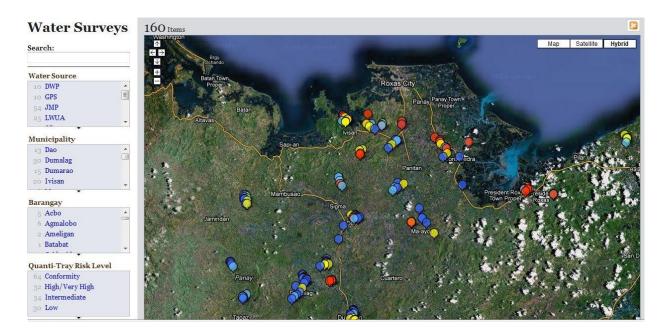


Figure 16: Water Quality Map of 160 Samples Collected in Capiz Province, Philippines

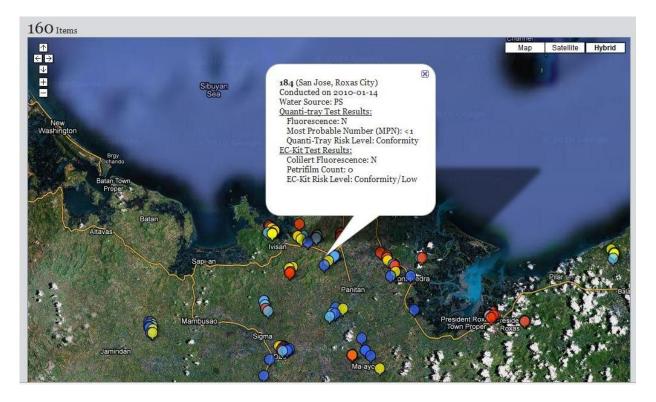


Figure 17: Detailed Water Quality Map Results Within a Data Point

The detailed map results may be viewed at the following website: <u>http://www.giswebsite.com/demos/PattyChuang04.html</u>.

This map is a contribution to a larger effort on the part of my advisor, Susan Murcott, to create a platform for a global drinking water quality map with crowd-source capability. A further dimension to this site is location-specific as well as country-by-country information on household drinking water treatment and safe storage solutions to address unsafe water supplies identified through the site-specific water quality data such as that above in Figure 16 and Figure 17.

CHAPTER 9: CONCLUSION AND RECOMMENDATIONS

9.1 EC-Kit Verification and Testing

From the chi-square and Fischer's exact tests, we see that each component of EC-Kit and the entire kit itself is correlated to Quanti-Tray® in a statistically significant way. Furthermore, from the calculations of error and proportional reduction in error for unimproved and improved water sources, it possible to make better predictions with just the use of the Colilert® test, but not just the use of the PetrifilmTM test. This is due to the detection limits for PetrifilmTM being much higher than Colilert®, namely positive PetrifilmTM results fall within the High and Very High risk level categories, whereas positive Colilert® results fall within the Intermediate, High, and Very High risk level categories. Most importantly, the two test set of the EC-Kit allows for the best reduction in error, with a proportional reduction in error of 63% for unimproved water sources and 60% for improved water sources.

9.2 Drinking Water Quality Mapping

The water quality mapping project is ongoing and the maps will be updated and modified in the future by the author, Jonathan Lowe, and Susan Murcott. The mapping coordinates were only as accurate as the GPS would allow (typical accuracy <15 meters feet range), especially in the rural areas of Capiz Province. Future updates to the map could allow users to input additional information, such as results from water quality tests beyond Quanti-Tray® and EC-Kit. Additionally, less technical information would be useful for users interested in mapping, such as displaying photos of the water source types, and short summaries of site-specific water sources.

9.3 Summary

Risk level data according to *Escherichia coli* and total coliform levels has been collected for drinking water sources for different locations and source types in Capiz Province. Quanti-Tray® results revealed that of the 521 water samples collected, none of the sources conformed to the WHO Guidelines for Drinking Water Quality. The details of each water sample may be found in Appendix B: Water Quality Results, Sorted by Municipality. Statistical analyses comparing the EC-Kit with Quanti-Tray® revealed that while the EC-Kit does not exactly match the Quanti-Tray® results, it still provides useful information for assessing at-risk water sources.

Furthermore, the basis for the Phlippines component of the global drinking water quality map has been created, and further modifications and updates to the map will be made in the future.

9.4 Recommendations for Future Studies

Due to the limitations present in the detection limits for Quanti-Tray® and PetrifilmTM, future projects verifying the EC-Kit are recommended to use Quanti-Tray@/2000.

In the author's field work in Capiz, one of the primary complications was with the field usage of the EC-Kit. Trainees of the EC-Kit attended a one-day workshop, and the training at this workshop was not complete enough for field tests to commence. The chief problem may have been that the training took place in May; however, due to unavoidable delays, the test program itself could not begin until December. Moreover, the initial version of the EC-Kit instructions lacked information regarding quality control procedures, and did not sufficiently emphasize the need to maintain sterile conditions when collecting water samples for the EC-Kit. Furthermore, there were no photos included with the instructions to help illustrate each step. Since then, the author and her thesis advisor have modified the EC-Kit instructions (see Appendix E: EC-Kit Instructions).

Future workshops in the EC-Kit should allow users to have a "trial" field testing, and a follow-up workshop to address any questions or confusion about the methods and interpretation of results. In particular, the author noticed that users of the EC-Kit were especially perplexed regarding how to properly perform the fluorescence readings for the Colilert® test and the PetrifilmTM gas-forming colony counts. The author advises future users of the EC-Kit to describe the Colilert® results as "milky, blue" fluorescence, and to emphasize reading PetrifilmTM results held up to proper daylight or bright lighting, so as to visibly notice the gas bubble formations. In addition, it is highly recommended that the PetrifilmTM *E.coli*/Coliform

Count Plate Interpretation Guide be utilized in analysis of PetrifilmTM results¹⁰. A possible method for future workshops could have trainees in pairs or small groups, and have each member individually count the blue colonies with gas bubbles, and have the group members compare their counts. This would highlight the need to only count the colonies with gas bubbles, not colonies without gas bubbles, and also introduce the concept of having a "double-check" count.

¹⁰ Document available for download under "Instructions for Use" at the 3M website: <u>http://solutions.3m.com/wps/portal/3M/en_US/Microbiology/FoodSafety/product-information/product-catalog/?PC_7_RJH9U523003DC023S7P92O3O87_nid=C0WJ62882Vbe29BDXSBJ7Fgl</u>

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APPENDICES

Appendix A: IDEXX 51-Well Quanti-Tray® MPN Table

IDEXX 51-Well Quanti-Tray	R MPN Table
Number of wells giving positive reaction	MPN per 100 mL sample
0	<1
1	1.0
2	2.0
3	3.1
4	4.2
5	5.3
6	6.4
7	7.5
8	8.7
9	9.9
10	11.1
11	12.4
12	13.7
13	15.0
14	16.4
15	17.8
16	19.2
17	20.7
18	22.2
19	23.8
20	25.4
21	27.1
22	28.8
23	30.6
24	32.4
25	34.4
26	36.4
27	38.4
28	40.6
29	42.9
30	45.3
31	47.8
32	50.4
33	53.1
34	56.0
35	59.1

36	62.4
37	65.9
38	69.7
39	73.8
40	78.2
41	83.1
42	88.5
43	94.5
44	101.3
45	109.1
46	118.4
47	129.8
48	144.5
49	165.2
50	200.5
51	200.5

Appendix B: Water Quality Results, Sorted by Municipality

Municipality	Sampling Date	Reading Date	Barangay Number	<i>Barangay</i> Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Cuartero	18-Jan-10	19-Jan-10	B4	Bitoon Ilaya	1	D	OD	Y	>200.5	Y	2.0
Cuartero	18-Jan-10	19-Jan-10	B3	Bitoon Ilawod	2	L1	JMP	Y	>200.5	Y	12.0
Cuartero	18-Jan-10	19-Jan-10	B3	Bitoon Ilawod	3	L1	JMP	Ν	<1	Ν	0.0
Cuartero	18-Jan-10	19-Jan-10	B3	Bitoon Ilawod	4	L1	JMP	Y	3.1	Y	0.0
Cuartero	18-Jan-10	19-Jan-10	B3	Bitoon Ilawod	5	L1	JMP	Y	1.0	Y	0.0
Cuartero	18-Jan-10	19-Jan-10	B3	Bitoon Ilawod	6	L1	JMP	Y	7.5	N	0.0
Cuartero	18-Jan-10	19-Jan-10	B9	Poblacion Ilawod	7	L1	JMP	Y	2.0	Y	0.0
Cuartero	18-Jan-10	19-Jan-10	B9	Poblacion Ilawod	8	L1	JMP	Ν	<1	Ν	0.0
Cuartero	18-Jan-10	19-Jan-10	B9	Poblacion Tacas	9	L1	SWP	Y	83.1	Y	0.0
Cuartero	18-Jan-10	19-Jan-10	B9	Poblacion Tacas	10	L1	JMP	Y	2.0	Ν	0.0
Cuartero	18-Jan-10	19-Jan-10	B9	Poblacion Tacas	11	L1	JMP	Ν	<1	Ν	0.0
Cuartero	18-Jan-10	19-Jan-10	B9	Poblacion Tacas	12	L1	JMP	Ν	<1	Ν	0.0
Cuartero	21-Jan-10	22-Jan-10	B10	Poblacion Ilaya	1	L1	JMP	Y	>200.5	Y	1.0
Cuartero	21-Jan-10	22-Jan-10	B10	Poblacion Ilaya	2	L1	JMP	Y	>200.5	Y	0.0
Cuartero	21-Jan-10	22-Jan-10	B10	Poblacion Ilaya	3	L1	JMP	Y	4.2	Ν	0.0
Cuartero	21-Jan-10	22-Jan-10	B10	Poblacion Ilaya	4	L1	JMP	Y	200.5	Y	0.0
Cuartero	21-Jan-10	22-Jan-10	B10	Poblacion Ilaya	5	L1	JMP	Y	>200.5	Y	18.0
Cuartero	21-Jan-10	22-Jan-10	B9	Poblacion Ilawod	6	L1	JMP	Ν	<1	Ν	0.0
Cuartero	21-Jan-10	22-Jan-10	B9	Poblacion Ilawod	7	L1	JMP	Ν	<1	Ν	0.0
Cuartero	21-Jan-10	22-Jan-10	B9	Poblacion Ilawod	8	L1	JMP	Y	1.0	Ν	0.0
Cuartero	2-Feb-10	3-Feb-10	B1	Agdahon	1	L2	GPS	Y	6.4	Y	0.0
Cuartero	2-Feb-10	3-Feb-10	B 1	Agdahon	2	L2	GPS	Ν	<1	Ν	0.0
Cuartero	2-Feb-10	3-Feb-10	B1	Agdahon	3	L2	GPS	Ν	<1	Ν	0.0
Cuartero	2-Feb-10	3-Feb-10	B1	Agdahon	4	L2	GPS	Y	1.0	Y	0.0

Municipality	Sampling Date	Reading Date	Barangay Number	<i>Barangay</i> Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Cuartero	2-Feb-10	3-Feb-10	B1	Agdahon	5	L2	GPS	Y	7.5	Ν	0.0
Cuartero	2-Feb-10	3-Feb-10	B5	Bun-od	6	L2	DWP	Y	11.1	Y	0.0
Cuartero	2-Feb-10	3-Feb-10	B5	Bun-od	7	L2	DWP	Ν	<1	Ν	0.0
Cuartero	2-Feb-10	3-Feb-10	B5	Bun-od	8	L2	DWP	Y	2.0	Ν	0.0
Cuartero	2-Feb-10	3-Feb-10	B5	Bun-od	9	L2	DWP	Ν	<1	Ν	0.0
Cuartero	2-Feb-10	3-Feb-10	B5	Bun-od	10	L2	DWP	Ν	<1	Ν	0.0
Dao	21-Jan-10	22-Jan-10	B5	Matagnop	1	L1	JMP	Ν	<1	Ν	0.0
Dao	21-Jan-10	22-Jan-10	B5	Matagnop	2	L1	JMP	Ν	<1	Ν	0.0
Dao	21-Jan-10	22-Jan-10	B5	Matagnop	3	L1	JMP	Ν	<1	Y	0.0
Dao	21-Jan-10	22-Jan-10	B5	Matagnop	4	L1	JMP	Y	5.3	Y	0.0
Dao	21-Jan-10	22-Jan-10	B5	Matagnop	5	L1	JMP	Y	>200.5	Y	0.0
Dao	21-Jan-10	22-Jan-10	B5	Matagnop	6	L1	JMP	Ν	<1	Y	0.0
Dao	21-Jan-10	22-Jan-10	B5	Matagnop	7	L1	JMP	Ν	<1	Ν	0.0
Dao	21-Jan-10	22-Jan-10	B5	Matagnop	8	L1	JMP	Ν	<1	Ν	0.0
Dao	21-Jan-10	22-Jan-10	B5	Matagnop	9	L1	SWP	Y	165.2	Y	6.0
Dao	21-Jan-10	22-Jan-10	B6	Nasunogan	10	L1	JMP	Y	19.2	Ν	0.0
Dao	21-Jan-10	22-Jan-10	B4	Poblacion Ilawod	11	L1	PDW	Y	34.4	Y	1.0
Dao	21-Jan-10	22-Jan-10	B4	Poblacion Ilawod	12	L1	PDW	Ν	<1	Ν	0.0
Dao	21-Jan-10	22-Jan-10	B7	Manhoy	13	L1	JMP	Ν	<1	Y	0.0
Dao	18-Jan-10	19-Jan-10	B3	Poblacion Ilaya	1	L1	JMP	Ν	<1	Ν	0.0
Dao	18-Jan-10	19-Jan-10	B3	Poblacion Ilaya	2	L1	JMP	Y	8.7	Ν	0.0
Dao	18-Jan-10	19-Jan-10	B3	Poblacion Ilaya	3	L1	JMP	Ν	<1	Ν	0.0
Dao	18-Jan-10	19-Jan-10	B3	Poblacion Ilaya	4	L1	JMP	Y	27.1	Y	0.0
Dao	18-Jan-10	19-Jan-10	B3	Poblacion Ilaya	5	L1	RW	Y	>200.5	Y	0.0
Dao	18-Jan-10	19-Jan-10	B4	Poblacion Ilawod	6	L1	RW	Y	>200.5	Y	8.0
Dao	18-Jan-10	19-Jan-10	B4	Poblacion Ilawod	7	L1	JMP	Ν	<1	Y	0.0
Dao	18-Jan-10	19-Jan-10	B4	Poblacion Ilawod	8	L1	JMP	Ν	<1	Ν	0.0

Municipality	Sampling Date	Reading Date	Barangay Number	Barangay Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Dao	18-Jan-10	19-Jan-10	B4	Poblacion Ilawod	9	L1	JMP	Y	2.0	Ν	0.0
Dao	18-Jan-10	19-Jan-10	B4	Poblacion Ilawod	10	L1	JMP	Ν	<1	Ν	0.0
Dao	18-Jan-10	19-Jan-10	B4	Poblacion Ilawod	11	L1	JMP	Y	>200.5	Ν	8.0
Dao	18-Jan-10	19-Jan-10	B4	Poblacion Ilawod	12	L1	JMP	Ν	<1	Y	0.0
Dao	16-Feb-10	17-Feb-10	B1	Balucuan	1	D	OT	Ν	<1	Ν	0.0
Dao	16-Feb-10	17-Feb-10	B1	Balucuan	2	D	OT	Y	6.4	Y	0.0
Dao	16-Feb-10	17-Feb-10	B1	Balucuan	3	D	OT	Ν	<1	Y	0.0
Dao	16-Feb-10	17-Feb-10	B1	Balucuan	4	D	OT	Ν	<1	Ν	0.0
Dumarao	19-Jan-10	20-Jan-10	B1	Codingle	1	L3	LWUA	Y	45.3	Y	0.0
Dumarao	19-Jan-10	20-Jan-10	B1	Codingle	2	L3	LWUA	Y	94.5	Y	0.0
Dumarao	19-Jan-10	20-Jan-10	B1	Codingle	3	L3	LWUA	Y	69.7	Y	0.0
Dumarao	19-Jan-10	20-Jan-10	B1	Codingle	4	L3	LWUA	Y	94.5	Y	2.0
Dumarao	19-Jan-10	20-Jan-10	B1	Codingle	5	L3	LWUA	Y	78.2	Y	3.0
Dumarao	19-Jan-10	20-Jan-10	B2	Poblacion Ilaya	6	L3	LWUA	Ν	<1	Ν	0.0
Dumarao	19-Jan-10	20-Jan-10	B2	Poblacion Ilaya	7	L3	LWUA	Ν	<1	Ν	0.0
Dumarao	19-Jan-10	20-Jan-10	B2	Poblacion Ilaya	8	L3	LWUA	Ν	<1	Y	0.0
Dumarao	19-Jan-10	20-Jan-10	B2	Poblacion Ilaya	9	L3	LWUA	Ν	<1	Ν	0.0
Dumarao	19-Jan-10	20-Jan-10	B2	Poblacion Ilaya	10	L3	LWUA	Y	3.1	Ν	0.0
Dumarao	19-Jan-10	20-Jan-10	B3	Poblacion Ilawod	11	L3	LWUA	Ν	<1	Ν	0.0
Dumarao	19-Jan-10	20-Jan-10	B3	Poblacion Ilawod	12	L3	LWUA	Ν	<1	Ν	0.0
Dumarao	19-Jan-10	20-Jan-10	B3	Poblacion Ilawod	13	L3	LWUA	Ν	<1	Ν	0.0
Dumarao	19-Jan-10	20-Jan-10	B3	Poblacion Ilawod	14	L3	LWUA	Ν	<1	Ν	0.0
Dumarao	19-Jan-10	20-Jan-10	B3	Poblacion Ilawod	15	L3	LWUA	Ν	<1	Ν	0.0
Dumarao	21-Jan-10	22-Jan-10	B1	Astorga	1	L1	SWP	Y	>200.5	Y	TNTC
Dumarao	21-Jan-10	22-Jan-10	B1	Astorga	2	L1	SWP	Y	>200.5	Y	12.0
Dumarao	21-Jan-10	22-Jan-10	B1	Astorga	3	L1	SWP	Y	42.9	Y	0.0
Dumarao	21-Jan-10	22-Jan-10	B2	Dacuton	4	L1	SWP	Y	3.1	Y	0.0

Municipality	Sampling Date	Reading Date	Barangay Number	Barangay Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Dumarao	21-Jan-10	22-Jan-10	B2	Dacuton	5	L1	SWP	Ν	<1	Ν	0.0
Dumarao	21-Jan-10	22-Jan-10	B3	Ongol Ilaya	6	L1	JMP	Y	>200.5	Y	TNTC
Dumarao	21-Jan-10	22-Jan-10	B3	Ongol Ilaya	7	L1	JMP	Y	>200.5	Y	3.0
Dumarao	21-Jan-10	22-Jan-10	B3	Ongol Ilaya	8	L1	JMP	Ν	<1	Ν	0.0
Dumarao	21-Jan-10	22-Jan-10	B3	Ongol Ilaya	9	L1	JMP	Ν	<1	Ν	0.0
Dumarao	21-Jan-10	22-Jan-10	B3	Ongol Ilaya	10	L1	JMP	Ν	<1	Ν	0.0
Dumarao	21-Jan-10	22-Jan-10	B3	Ongol Ilaya	11	L1	JMP	Y	165.2	Y	0.0
Dumarao	21-Jan-10	22-Jan-10	B3	Ongol Ilaya	12	L1	JMP	Y	200.5	Y	3.0
Dumarao	18-Jan-10	19-Jan-10	B1	Tinatayan	1	L1	JMP	Y	5.3	Ν	0.0
Dumarao	18-Jan-10	19-Jan-10	B1	Tinatayan	2	L1	JMP	Ν	<1	Ν	0.0
Dumarao	18-Jan-10	19-Jan-10	B1	Tinatayan	3	L1	JMP	Ν	<1	Ν	0.0
Dumarao	18-Jan-10	19-Jan-10	B1	Tinatayan	4	L1	JMP	Ν	<1	Ν	0.0
Dumarao	18-Jan-10	19-Jan-10	B1	Tinatayan	5	L1	JMP	Y	5.3	Y	0.0
Dumarao	18-Jan-10	19-Jan-10	B1	Tinatayan	6	L1	JMP	Ν	<1	Y	0.0
Dumarao	18-Jan-10	19-Jan-10	B1	Tinatayan	7	L1	JMP	Y	>200.5	Y	1.0
Dumarao	18-Jan-10	19-Jan-10	B2	Ongal Ilawod	1	L1	JMP	Ν	<1	Ν	0.0
Dumarao	18-Jan-10	19-Jan-10	B2	Ongal Ilawod	2	L1	JMP	Ν	<1	Ν	0.0
Dumarao	18-Jan-10	19-Jan-10	B2	Ongal Ilawod	3	L1	JMP	Ν	<1	Ν	0.0
Dumarao	18-Jan-10	19-Jan-10	B2	Ongal Ilawod	4	L1	JMP	Y	3.1	Ν	0.0
Dumarao	18-Jan-10	19-Jan-10	B2	Ongal Ilawod	5	L1	JMP	Ν	<1	Ν	0.0
Dumarao	18-Jan-10	19-Jan-10	B2	Ongal Ilawod	6	L1	JMP	Ν	<1	Ν	0.0
Dumarao	2-Feb-10	3-Feb-10	B1	Aglalana	1	L2	GPS	Y	69.7	Y	0.0
Dumarao	2-Feb-10	3-Feb-10	B1	Aglalana	2	L2	GPS	Y	69.7	Y	2.0
Dumarao	2-Feb-10	3-Feb-10	B1	Aglalana	3	L2	GPS	Y	83.1	Y	1.0
Dumarao	2-Feb-10	3-Feb-10	B1	Aglalana	4	L2	GPS	Y	63.1	Y	3.0
Dumarao	2-Feb-10	3-Feb-10	B1	Aglalana	5	L2	GPS	Ν	<1	Ν	0.0
Dumarao	16-Feb-10	17-Feb-10	B1	Salcedo	1	D	OD	Y	1.0	Ν	0.0

Municipality	Sampling Date	Reading Date	Barangay Number	Barangay Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Dumarao	16-Feb-10	17-Feb-10	B1	Salcedo	2	D	OT	Ν	<1	Y	0.0
Dumarao	16-Feb-10	17-Feb-10	B1	Salcedo	3	D	US	Y	>200.5	Y	1.0
Dumarao	16-Feb-10	17-Feb-10	B1	Salcedo	4	D	OT	Y	>200.5	Y	18.0
Dumarao	16-Feb-10	17-Feb-10	B1	Salcedo	5	D	SW	Ν	<1	Ν	0.0
Dumalag	12-Jan-10	13-Jan-10	SC	Santa Cruz	1	L1	JMP	Ν	<1	Ν	1.0
Dumalag	12-Jan-10	13-Jan-10	SC	Santa Cruz	2	L1	JMP	Y	11.1	Ν	0.0
Dumalag	12-Jan-10	13-Jan-10	SC	Santa Cruz	3	L1	JMP	Y	32.4	Y	11.0
Dumalag	12-Jan-10	13-Jan-10	SC	Santa Cruz	4	L1	JMP	Y	1.0	Ν	1.0
Dumalag	12-Jan-10	13-Jan-10	SC	Santa Cruz	5	L1	JMP	Ν	<1	Ν	1.0
Dumalag	12-Jan-10	13-Jan-10	SC	Santa Cruz	6	L1	JMP	Ν	<1	Ν	1.0
Dumalag	12-Jan-10	13-Jan-10	SC	Santa Cruz	7	L1	JMP	Y	129.8	Y	11.0
Dumalag	12-Jan-10	13-Jan-10	SC	Santa Cruz	8	L1	JMP	Ν	<1	Ν	2.0
Dumalag	12-Jan-10	13-Jan-10	SR	San Roque	1	L1	JMP	N	<1	Y	0.0
Dumalag	12-Jan-10	13-Jan-10	SR	San Roque	2	L1	JMP	Ν	<1	Y	2.0
Dumalag	12-Jan-10	13-Jan-10	SR	San Roque	3	L1	JMP	Y	15.0	Y	11.0
Dumalag	12-Jan-10	13-Jan-10	SR	San Roque	4	L1	JMP	Y	16.0	Y	11.0
Dumalag	12-Jan-10	13-Jan-10	SR	San Roque	5	L1	JMP	Y	1.0	Ν	1.0
Dumalag	12-Jan-10	13-Jan-10	SR	San Roque	6	L1	JMP	Ν	<1	Ν	0.0
Dumalag	12-Jan-10	13-Jan-10	SR	San Roque	7	L1	JMP	Ν	<1	Ν	0.0
Dumalag	13-Jan-10	14-Jan-10	BD	Dolores	1	L3	LWUA	Y	45.0	Ν	0.0
Dumalag	13-Jan-10	14-Jan-10	BD	Dolores	2	L3	LWUA	Ν	<1	Ν	0.0
Dumalag	13-Jan-10	14-Jan-10	BD	Dolores	3	L3	LWUA	Ν	<1	Ν	0.0
Dumalag	13-Jan-10	14-Jan-10	BD	Dolores	4	L3	LWUA	Y	1.0	Ν	0.0
Dumalag	13-Jan-10	14-Jan-10	BD	Dolores	5	L3	LWUA	Ν	<1	Ν	0.0
Dumalag	13-Jan-10	14-Jan-10	BP	Poblacion	1	L3	LWUA	Ν	<1	Ν	0.0
Dumalag	13-Jan-10	14-Jan-10	BP	Poblacion	2	L3	LWUA	Ν	<1	Y	0.0
Dumalag	13-Jan-10	14-Jan-10	BP	Poblacion	3	L3	LWUA	Ν	<1	Ν	0.0

Municipality	Sampling Date	Reading Date	Barangay Number	<i>Barangay</i> Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Dumalag	13-Jan-10	14-Jan-10	BP	Poblacion	4	L3	LWUA	Ν	<1	Ν	2.0
Dumalag	13-Jan-10	14-Jan-10	BP	Poblacion	5	L3	LWUA	Ν	<1	Ν	0.0
Dumalag	13-Jan-10	14-Jan-10	BSA	San Angel	1	D	OD	Y	>200.5	Y	6.0
Dumalag	13-Jan-10	14-Jan-10	BSA	San Angel	2	D	OD	Y	165.2	Y	0.0
Dumalag	13-Jan-10	14-Jan-10	BSA	San Angel	3	D	OD	Y	>200.5	Y	7.0
Dumalag	13-Jan-10	14-Jan-10	BSA	San Angel	4	D	OD	Y	>200.5	Y	TNTC
Dumalag	13-Jan-10	14-Jan-10	BC	Concepcion	1	L1	PS	Ν	<1	Ν	0.0
Tapaz	20-Jan-10	21-Jan-10	B6	San Julian	1	L2	DWP	Y	8.7	Ν	0.0
Tapaz	20-Jan-10	21-Jan-10	B6	San Julian	2	L2	DWP	Ν	<1	Ν	0.0
Tapaz	20-Jan-10	21-Jan-10	B6	San Julian	3	L2	DWP	Ν	<1	Ν	0.0
Tapaz	20-Jan-10	21-Jan-10	B6	San Julian	4	L2	DWP	Y	1.0	Ν	0.0
Tapaz	20-Jan-10	21-Jan-10	B6	San Julian	5	L2	DWP	Ν	<1	Ν	0.0
Tapaz	20-Jan-10	21-Jan-10	B7	San Nicolas	1	L2	GPS	Ν	<1	Ν	0.0
Tapaz	20-Jan-10	21-Jan-10	B7	San Nicolas	2	L2	GPS	Ν	<1	Ν	0.0
Tapaz	20-Jan-10	21-Jan-10	B7	San Nicolas	3	L2	GPS	Ν	<1	Ν	0.0
Tapaz	20-Jan-10	21-Jan-10	B7	San Nicolas	4	L2	GPS	Ν	<1	Ν	0.0
Tapaz	20-Jan-10	21-Jan-10	B7	San Nicolas	5	L2	GPS	Y	2.0	Ν	0.0
Tapaz	12-Jan-10	13-Jan-10	B1	Salong	1	L1	PDW	Y	>200.5	Y	5.0
Tapaz	12-Jan-10	13-Jan-10	B1	Salong	6	L1	JMP	Y	<1	Ν	0.0
Tapaz	12-Jan-10	13-Jan-10	B1	Salong	11	L1	JMP	Y	9.9	Y	0.0
Tapaz	12-Jan-10	13-Jan-10	B1	Salong	12	L1	JMP	Y	2.0	Ν	0.0
Tapaz	12-Jan-10	13-Jan-10	B1	Salong	13	L1	DWP	Y	1.0	Ν	0.0
Tapaz	12-Jan-10	13-Jan-10	B2	Carida	3	L1	JMP	Y	165.2	Y	1.0
Tapaz	12-Jan-10	13-Jan-10	B2	Carida	4	L1	JMP	Y	2.0	Ν	0.0
Tapaz	12-Jan-10	13-Jan-10	B2	Carida	5	L1	JMP	Y	>200.5	Y	4.0
Tapaz	12-Jan-10	13-Jan-10	B2	Carida	7	L1	JMP	Y	5.3	Y	2.0
Tapaz	12-Jan-10	13-Jan-10	B2	Carida	8	L1	DWP	Y	17.8	Y	1.0

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Tapaz	12-Jan-10	13-Jan-10	B2	Carida	9	L1	DWP	Y	25.4	Y	0.0
Tapaz	12-Jan-10	13-Jan-10	B2	Carida	10	L1	JMP	Y	>200.5	Y	3.0
Tapaz	12-Jan-10	13-Jan-10	B3	Poblacion	2	L1	DWP	Y	23.8	Y	1.0
Tapaz	12-Jan-10	13-Jan-10	B3	Poblacion	14	L1	DWP	Y	25.4	Y	2.0
Tapaz	12-Jan-10	13-Jan-10	B3	Poblacion	21	L1	JMP	Y	>200.5	Y	4.0
Tapaz	12-Jan-10	13-Jan-10	B3	Poblacion	22	L1	JMP	Y	>200.5	Y	7.0
Tapaz	12-Jan-10	13-Jan-10	B3	Poblacion	23	L1	JMP	Y	7.5	Ν	0.0
Tapaz	12-Jan-10	13-Jan-10	B3	Poblacion	24	L1	JMP	Y	4.2	Ν	0.0
Tapaz	12-Jan-10	13-Jan-10	B3	Poblacion	25	L1	JMP	Y	2.0	Y	0.0
Tapaz	12-Jan-10	13-Jan-10	B4	San Jose	16	L1	JMP	Y	>200.5	Y	8.0
Tapaz	12-Jan-10	13-Jan-10	B4	San Jose	17	L1	JMP	Y	>200.5	Y	TNTC
Tapaz	12-Jan-10	13-Jan-10	B4	San Jose	18	L1	JMP	Y	165.2	Y	0.0
Tapaz	12-Jan-10	13-Jan-10	B4	San Jose	19	L1	JMP	Y	>200.5	Y	14.0
Tapaz	12-Jan-10	13-Jan-10	B4	San Jose	20	L1	JMP	Y	>200.5	Y	3.0
Tapaz	12-Jan-10	13-Jan-10	B5	Cagoungan	15	L1	JMP	Y	>200.5	Y	5.0
Tapaz	9-Feb-10	10-Feb-10	B8	Camburanan	1	L2	GPS	Y	8.7	Y	0.0
Tapaz	9-Feb-10	10-Feb-10	B8	Camburanan	2	L2	GPS	Y	7.5	Ν	0.0
Tapaz	9-Feb-10	10-Feb-10	B8	Camburanan	3	L2	GPS	Y	8.7	Y	0.0
Tapaz	9-Feb-10	10-Feb-10	B8	Camburanan	4	L2	GPS	Y	36.4	Y	0.0
Tapaz	9-Feb-10	10-Feb-10	B8	Camburanan	5	L2	GPS	Y	73.8	Y	0.0
Ivisan	17-Mar-10	18-Mar-10	B1	Santa Cruz	1	L1	JMP	Y	>200.5	Y	16.0
Ivisan	17-Mar-10	18-Mar-10	B1	Santa Cruz	2	L1	JMP	Y	>200.5	Y	12.0
Ivisan	17-Mar-10	18-Mar-10	B1	Santa Cruz	3	L1	JMP	Y	15.0	Ν	0.0
Ivisan	17-Mar-10	18-Mar-10	B1	Santa Cruz	4	L1	JMP	Y	101.3	Y	3.0
Ivisan	17-Mar-10	18-Mar-10	B1	Santa Cruz	5	L1	JMP	Y	4.2	Ν	0.0
Ivisan	17-Mar-10	18-Mar-10	B2	Ilaya	6	L1	JMP	Y	4.2	Ν	0.0
Ivisan	17-Mar-10	18-Mar-10	B2	Ilaya	7	L1	JMP	Y	17.8	Y	0.0

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Ivisan	17-Mar-10	18-Mar-10	B2	Ilaya	8	L1	JMP	Y	17.8	Y	0.0
Ivisan	17-Mar-10	18-Mar-10	B2	Ilaya	9	L1	JMP	Ν	<1	Ν	0.0
Ivisan	17-Mar-10	18-Mar-10	B2	Ilaya	10	L1	JMP	Y	15.0	Ν	0.0
Ivisan	24-Mar-10	25-Mar-10	B3	Matnog	1	L2	GPS	Ν	<1	Ν	0.0
Ivisan	24-Mar-10	25-Mar-10	B3	Matnog	2	L2	GPS	Y	6.4	Ν	0.0
Ivisan	24-Mar-10	25-Mar-10	B4	Agmalobo	3	D	US	Y	1.0	Y	0.0
Ivisan	24-Mar-10	25-Mar-10	B4	Agmalobo	4	D	US	Y	4.2	Y	0.0
Maayon	8-Jan-10	9-Jan-10	B1	Quinat-Uyan	6	L1	JMP	Ν	<1	Y	0.0
Maayon	8-Jan-10	9-Jan-10	B2	Badabat	6	L1	JMP	Y	83.1	Y	0.0
Maayon	8-Jan-10	9-Jan-10	B4	Palaguian	6	L1	JMP	Ν	<1	Y	0.0
Maayon	8-Jan-10	9-Jan-10	B5	Ilawod	1	D	OD	Y	>200.5	Y	9.0
Maayon	8-Jan-10	9-Jan-10	B5	Ilawod	2	D	OD	Y	25.4	Y	0.0
Maayon	8-Jan-10	9-Jan-10	B5	Ilawod	3	D	OD	Y	8.7	Y	1.0
Maayon	8-Jan-10	9-Jan-10	B6	Tabuc	1	L1	SWP	Ν	<1	Ν	0.0
Maayon	8-Jan-10	9-Jan-10	B6	Tabuc	2	L1	SWP	Ν	<1	Ν	0.0
Maayon	7-Jan-10	8-Jan-10	B3	Cabungahan	1	L1	JMP	Y	59.1	Y	1.0
Maayon	7-Jan-10	8-Jan-10	B3	Cabungahan	2	L1	JMP	Y	83.1	Y	1.0
Maayon	7-Jan-10	8-Jan-10	B3	Cabungahan	3	L1	JMP	Ν	<1	Y	0.0
Maayon	7-Jan-10	8-Jan-10	B3	Cabungahan	4	L1	JMP	Y	3.1	Ν	0.0
Maayon	7-Jan-10	8-Jan-10	B3	Cabungahan	5	L1	JMP	Y	6.4	Y	0.0
Maayon	7-Jan-10	8-Jan-10	B4	Palaguian	1	L1	JMP	Ν	<1	Ν	0.0
Maayon	7-Jan-10	8-Jan-10	B4	Palaguian	2	L1	JMP	Y	5.3	Y	0.0
Maayon	7-Jan-10	8-Jan-10	B4	Palaguian	3	L1	JMP	Y	>200.5	Y	6.0
Maayon	7-Jan-10	8-Jan-10	B4	Palaguian	4	L1	JMP	Ν	<1	Ν	0.0
Maayon	7-Jan-10	8-Jan-10	B4	Palaguian	5	L1	JMP	Y	3.1	Ν	0.0
Maayon	10-Dec-09	11-Dec-09	B2	Batabat	1	L1	JMP	Y	2.0	Y	0.0
Maayon	10-Dec-09	11-Dec-09	B2	Batabat	2	L1	JMP	Y	1.0	Ν	0.0

Municipality	Sampling Date	Reading Date	Barangay Number	<i>Barangay</i> Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Maayon	10-Dec-09	11-Dec-09	B2	Batabat	3	L1	JMP	Ν	<1	Ν	0.0
Maayon	10-Dec-09	11-Dec-09	B2	Batabat	4	L1	JMP	Ν	<1	Ν	0.0
Maayon	10-Dec-09	11-Dec-09	B2	Batabat	5	L1	JMP	Y	>200.5	Y	1.0
Maayon	10-Dec-09	11-Dec-09	B1	Quinat-Uyan	1	L1	JMP	Y	200.5	Y	1.0
Maayon	10-Dec-09	11-Dec-09	B1	Quinat-Uyan	2	L1	JMP	Y	27.1	Ν	0.0
Maayon	10-Dec-09	11-Dec-09	B1	Quinat-Uyan	3	L1	JMP	Y	>200.5	Y	7.0
Maayon	10-Dec-09	11-Dec-09	B1	Quinat-Uyan	4	L1	JMP	Y	42.9	Y	2.0
Maayon	10-Dec-09	11-Dec-09	B1	Quinat-Uyan	5	L1	JMP	Y	12.4	Y	0.0
Maayon	4-Feb-10	5-Feb-10	B7	Poblacion Ilaya	1	L2	DWP	Y	129.8	Y	1.0
Maayon	4-Feb-10	5-Feb-10	B7	Poblacion Ilaya	2	L2	DWP	Y	23.8	Y	0.0
Maayon	4-Feb-10	5-Feb-10	B7	Poblacion Ilaya	3	L2	DWP	Y	25.4	Y	0.0
Maayon	4-Feb-10	5-Feb-10	B7	Poblacion Ilaya	4	L2	DWP	Y	23.8	Ν	0.0
Maayon	4-Feb-10	5-Feb-10	B7	Poblacion Ilaya	5	L2	DWP	Ν	<1	Ν	0.0
Maayon	4-Feb-10	5-Feb-10	B8	Tapulang	1	L2	DWP	Y	6.4	Ν	0.0
Maayon	4-Feb-10	5-Feb-10	B8	Tapulang	2	L2	DWP	Y	16.4	Y	0.0
Maayon	4-Feb-10	5-Feb-10	B8	Tapulang	3	L2	DWP	Y	6.4	Y	0.0
Maayon	4-Feb-10	5-Feb-10	B8	Tapulang	4	L2	DWP	Y	19.2	Y	0.0
Maayon	4-Feb-10	5-Feb-10	B8	Tapulang	5	L2	DWP	Y	1.0	Y	12.0
Maayon	4-Feb-10	5-Feb-10	B5	Poblacion Ilawod	4	D	US	Y	38.4	Ν	0.0
Maayon	4-Feb-10	5-Feb-10	B5	Poblacion Ilawod	5	D	OD	Y	>200.5	Y	10.0
Pilar	8-Jan-10	9-Jan-10	B4	San Pedro	1	D	OD	Y	2.0	Y	0.0
Pilar	8-Jan-10	9-Jan-10	B4	San Pedro	2	D	OD	Y	83.1	Y	0.0
Pilar	8-Jan-10	9-Jan-10	B4	San Pedro	3	D	OD	Y	32.4	Y	0.0
Pilar	8-Jan-10	9-Jan-10	B4	San Pedro	4	D	OD	Y	62.4	Ν	2.0
Pilar	8-Jan-10	9-Jan-10	B4	San Pedro	5	D	OD	Y	7.5	Ν	1.0
Pilar	7-Jan-10	8-Jan-10	B3	Poblacion	1	L1	JMP	Y	12.4	Y	3.0
Pilar	7-Jan-10	8-Jan-10	B3	Poblacion	2	L1	JMP	Y	>200.5	Y	8.0

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Pilar	7-Jan-10	8-Jan-10	B3	Poblacion	3	L1	JMP	Y	>200.5	Ν	1.0
Pilar	7-Jan-10	8-Jan-10	B3	Poblacion	4	L1	JMP	Y	5.3	Y	0.0
Pilar	7-Jan-10	8-Jan-10	B3	Poblacion	5	L1	JMP	Y	200.5	Y	1.0
Pilar	7-Jan-10	8-Jan-10	B3	Poblacion	6	L1	JMP	Y	<1	Y	0.0
Pilar	7-Jan-10	8-Jan-10	B3	Poblacion	7	L1	JMP	Y	4.2	Ν	0.0
Pilar	7-Jan-10	8-Jan-10	B3	Poblacion	8	L1	JMP	Y	6.4	Y	0.0
Pilar	7-Jan-10	8-Jan-10	B3	Poblacion	9	L1	JMP	Ν	<1	Ν	0.0
Pilar	7-Jan-10	8-Jan-10	B3	Poblacion	10	L1	JMP	Y	<1	Ν	0.0
Pilar	10-Dec-09	11-Dec-09	B1	Natividad	1	L1	JMP	Y	3.1	Y	0.0
Pilar	10-Dec-09	11-Dec-09	B1	Natividad	2	L1	JMP	Ν	<1	Ν	0.0
Pilar	10-Dec-09	11-Dec-09	B1	Natividad	3	L1	JMP	Y	5.3	Ν	0.0
Pilar	10-Dec-09	11-Dec-09	B1	Natividad	4	L1	JMP	Y	6.4	Y	1.0
Pilar	10-Dec-09	11-Dec-09	B1	Natividad	5	L1	JMP	Y	>200.5	Y	50.0
Pilar	10-Dec-09	11-Dec-09	B2	Cayos	1	L1	JMP	Y	>200.5	Y	7.0
Pilar	10-Dec-09	11-Dec-09	B2	Cayos	2	L1	JMP	Y	>200.5	Y	11.0
Pilar	10-Dec-09	11-Dec-09	B2	Cayos	3	L1	JMP	Y	>200.5	Y	18.0
Pilar	10-Dec-09	11-Dec-09	B2	Cayos	4	L1	JMP	Ν	<1	Ν	0.0
Pilar	10-Dec-09	11-Dec-09	B2	Cayos	5	L1	JMP	Y	>200.5	Y	12.0
Pilar	18-Feb-10	19-Feb-10	B6	Poblacion	1	L3	LWUA	Y	5.3	Y	0.0
Pilar	18-Feb-10	19-Feb-10	B7	San Esteban	2	L3	LWUA	Y	8.7	Ν	0.0
Pilar	18-Feb-10	19-Feb-10	B7	San Esteban	3	L3	LWUA	Y	2.0	Y	0.0
Pilar	18-Feb-10	19-Feb-10	B7	San Esteban	4	L3	LWUA	Y	1.0	Y	0.0
Pilar	18-Feb-10	19-Feb-10	B7	San Esteban	5	L3	LWUA	Y	1.0	Y	0.0
Pilar	18-Feb-10	19-Feb-10	B7	San Esteban	6	L3	LWUA	Y	4.2	Ν	0.0
Pilar	18-Feb-10	19-Feb-10	B7	San Esteban	7	L3	LWUA	Ν	<1	Ν	0.0
Pilar	18-Feb-10	19-Feb-10	B7	San Esteban	8	L3	LWUA	Y	2.0	Y	0.0
Pilar	18-Feb-10	19-Feb-10	B7	San Esteban	9	L3	LWUA	Y	3.1	Y	0.0

Municipality	Sampling Date	Reading Date	Barangay Number	Barangay Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Pilar	18-Feb-10	19-Feb-10	B7	San Esteban	10	L3	LWUA	Y	2.0	Ν	0.0
Pilar	18-Feb-10	19-Feb-10	B7	San Esteban	11	L3	LWUA	Y	5.3	Ν	0.0
Pilar	18-Feb-10	19-Feb-10	B6	Poblacion	12	L3	LWUA	Y	11.1	Ν	0.0
Pilar	18-Feb-10	19-Feb-10	B6	Poblacion	13	L3	LWUA	Y	7.5	Ν	1.0
Pilar	18-Feb-10	19-Feb-10	B6	Poblacion	14	L3	LWUA	Y	2.0	Ν	0.0
Pilar	18-Feb-10	19-Feb-10	B6	Poblacion	15	L3	LWUA	Y	3.1	Y	0.0
Pontevedra	7-Jan-10	8-Jan-10	B20	Rizal	10	L1	JMP	N	<1	N	0.0
Pontevedra	7-Jan-10	8-Jan-10	B23	Nelia Manaay	4	L1	JMP	Ν	<1	Y	0.0
Pontevedra	7-Jan-10	8-Jan-10	B10	Guba	8	L1	JMP	Y	>200.5	N	0.0
Pontevedra	7-Jan-10	8-Jan-10	B25	Tacas	9	L1	SWP	N	<1	N	0.0
Pontevedra	7-Jan-10	8-Jan-10	B3	Ameligan	1	L1	RW	Y	>200.5	Y	3.0
Pontevedra	7-Jan-10	8-Jan-10	B3	Ameligan	2	L1	RW	Y	5.3	N	0.0
Pontevedra	7-Jan-10	8-Jan-10	B10	Guba	6	D	OD	N	<1	N	0.0
Pontevedra	7-Jan-10	8-Jan-10	B10	Guba	7	D	OD	Y	2.0	N	0.0
Pontevedra	7-Jan-10	8-Jan-10	B23	Nelia Manaay	3	D	OD	Y	30.6	Y	0.0
Pontevedra	7-Jan-10	8-Jan-10	B10	Guba	5	D	OD	Y	>200.5	Y	6.0
Pontevedra	10-Dec-09	11-Dec-09	B23	Sublangon	1	L1	JMP	N	<1	N	0.0

Municipality	Sampling Date	Reading Date	Barangay Number	Barangay Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Pontevedra	10-Dec-09	11-Dec-09	B25	Tacas	2	L1	JMP	N	<1	N	0.0
Pontevedra	10-Dec-09	11-Dec-09	B25	Tacas	3	L1	JMP	Y	47.8	Y	0.0
Pontevedra	10-Dec-09	11-Dec-09	B25	Tacas	4	L1	SWP	Y	1.0	N	0.0
Pontevedra	10-Dec-09	11-Dec-09	B20	Rizal	5	L1	JMP	N	<1	N	0.0
Pontevedra	10-Dec-09	11-Dec-09	B15	Jolongajog	6	L1	JMP	Y	>200.5	Y	1.0
Pontevedra	10-Dec-09	11-Dec-09	B7	Binuntucan	7	L1	JMP	Y	101.3	Y	0.0
Pontevedra	10-Dec-09	11-Dec-09	B7	Binuntucan	8	L1	JMP	Y	200.5	Y	2.0
Pontevedra	10-Dec-09	11-Dec-09	B7	Binuntucan	9	L1	JMP	Y	>200.5	Y	46.0
Pontevedra	10-Dec-09	11-Dec-09	B7	Binuntucan	10	L1	JMP	Y	>200.5	Y	4.0
Pontevedra	4-Feb-10	5-Feb-10	B4	Bailan	1	L2	DWP	Y	2.0	N	0.0
Pontevedra	4-Feb-10	5-Feb-10	B4	Bailan	2	L2	DWP	Y	4.2	N	0.0
Pontevedra	4-Feb-10	5-Feb-10	B4	Bailan	3	L2	DWP	Y	7.5	N	0.0
Pontevedra	4-Feb-10	5-Feb-10	B4	Bailan	4	L2	DWP	Y	2.0	N	0.0
Pontevedra	4-Feb-10	5-Feb-10	B4	Bailan	5	L2	DWP	Y	>200.5	Y	4.0

Municipality	Sampling Date	Reading Date	Barangay Number	Barangay Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Pontevedra	4-Feb-10	5-Feb-10	B15	Jolongajog	6	L1	JMP	N	<1	N	0.0
Pontevedra	4-Feb-10	5-Feb-10	B15	Jolongajog	7	L1	JMP	Y	165.2	Y	1.0
Pontevedra	4-Feb-10	5-Feb-10	B15	Jolongajog	8	L1	JMP	Y	>200.5	Y	TNTC
Pontevedra	4-Feb-10	5-Feb-10	B7	Binuntucan	9	L1	JMP	Y	4.2	N	0.0
Pontevedra	4-Feb-10	5-Feb-10	B7	Binuntucan	10	L1	JMP	Y	>200.5	Y	10.0
Pontevedra	18-Feb-10	19-Feb-10	B13	Ilawod	1	L3	LWUA	N	<1	N	0.0
Pontevedra	18-Feb-10	19-Feb-10	B13	Ilawod	2	L3	LWUA	Y	6.4	N	0.0
Pontevedra	18-Feb-10	19-Feb-10	B13	Ilawod	3	L3	LWUA	Y	30.6	N	0.0
Pontevedra	18-Feb-10	19-Feb-10	B13	Ilawod	4	L3	LWUA	Y	3.1	N	0.0
Pontevedra	18-Feb-10	19-Feb-10	B13	Ilawod	5	L3	LWUA	N	<1	N	0.0
Pontevedra	18-Feb-10	19-Feb-10	B11	Hipona	6	L3	LWUA	N	<1	N	0.0
Pontevedra	18-Feb-10	19-Feb-10	B11	Hipona	7	L3	LWUA	Y	1.0	N	0.0
Pontevedra	18-Feb-10	19-Feb-10	B11	Hipona	8	L3	LWUA	N	<1	N	0.0
Pontevedra	18-Feb-10	19-Feb-10	B11	Hipona	9	L3	LWUA	Y	1.0	N	0.0

Municipality	Sampling Date	Reading Date	Barangay Number	<i>Barangay</i> Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Pontevedra	18-Feb-10	19-Feb-10	B11	Hipona	10	L3	LWUA	Ν	<1	N	0.0
Roxas City	14-Jan-10	15-Jan-10	B1	Lanot	1	L1	SWP	Y	>200.5	Y	TNTC
Roxas City	14-Jan-10	15-Jan-10	B1	Lanot	2	L1	SWP	Ν	<1	Ν	0.0
Roxas City	14-Jan-10	15-Jan-10	B1	Lanot	3	L1	SWP	Y	>200.5	Y	5.0
Roxas City	14-Jan-10	15-Jan-10	B1	Lanot	4	L1	SWP	Ν	<1	Ν	0.0
Roxas City	14-Jan-10	15-Jan-10	B2	San Jose	5	L1	PS	Ν	<1	Ν	0.0
Roxas City	14-Jan-10	15-Jan-10	B2	San Jose	6	D	OD	Y	15.0	Y	0.0
Roxas City	14-Jan-10	15-Jan-10	B3	Jumaquicjic	7	L1	SWP	Ν	<1	Ν	0.0
Roxas City	14-Jan-10	15-Jan-10	B3	Jumaquicjic	8	L1	SWP	Ν	<1	Ν	0.0
Roxas City	14-Jan-10	15-Jan-10	B3	Jumaquicjic	9	L1	SWP	Y	1.0	Ν	0.0
Roxas City	14-Jan-10	15-Jan-10	B3	Jumaquicjic	10	L1	SWP	Ν	<1	Ν	0.0
Roxas City	5-Jan-10	6-Jan-10	B1	Laxa-an	1	L1	PDW	Y	>200.5	Y	TNTC
Roxas City	5-Jan-10	6-Jan-10	B1	Laxa-an	2	L1	JMP	Ν	<1	Y	0.0
Roxas City	5-Jan-10	6-Jan-10	B1	Laxa-an	3	L1	JMP	Y	>200.5	Y	TNTC
Roxas City	5-Jan-10	6-Jan-10	B1	Laxa-an	4	L1	JMP	Y	>200.5	Y	TNTC
Roxas City	5-Jan-10	6-Jan-10	B1	Laxa-an	5	L1	JMP	Ν	<1	Ν	0.0
Roxas City	5-Jan-10	6-Jan-10	B1	Laxa-an	6	L1	JMP	Ν	<1	Ν	0.0
Roxas City	5-Jan-10	6-Jan-10	B1	Laxa-an	7	L1	JMP	Y	129.8	Y	2.0
Roxas City	5-Jan-10	6-Jan-10	B2	Sibaguan	1	L1	JMP	Ν	<1	Ν	0.0
Roxas City	5-Jan-10	6-Jan-10	B2	Sibaguan	2	L1	JMP	Y	129.1	Y	4.0
Roxas City	5-Jan-10	6-Jan-10	B2	Sibaguan	3	L1	JMP	Y	200.5	Y	15.0
Roxas City	5-Jan-10	6-Jan-10	B2	Sibaguan	4	L1	JMP	Y	129.8	Y	4.0
Roxas City	5-Jan-10	6-Jan-10	B2	Sibaguan	5	L1	JMP	Y	3.1	Y	0.0
Roxas City	5-Jan-10	6-Jan-10	B3	Lonoy	1	L1	JMP	Y	1.0	Ν	0.0
Roxas City	5-Jan-10	6-Jan-10	B3	Lonoy	2	L1	JMP	Y	1.0	Ν	0.0
Roxas City	5-Jan-10	6-Jan-10	B3	Lonoy	3	L1	JMP	Y	47.8	Y	1.0

Municipality	Sampling Date	Reading Date	Barangay Number	Barangay Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Roxas City	5-Jan-10	6-Jan-10	B3	Lonoy	4	L1	JMP	Ν	<1	Ν	0.0
Roxas City	5-Jan-10	6-Jan-10	B4	Liong	1	L1	JMP	Ν	<1	Y	0.0
Roxas City	5-Jan-10	6-Jan-10	B4	Liong	2	L1	JMP	Y	118.4	Y	0.0
Roxas City	5-Jan-10	6-Jan-10	B4	Liong	3	L1	JMP	Y	200.5	Y	3.0
Roxas City	5-Jan-10	6-Jan-10	B4	Liong	4	L1	JMP	Ν	<1	Ν	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	1	L1	JMP	Ν	<1	Ν	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	2	L1	JMP	Y	1.0	Ν	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	3	L1	JMP	Y	12.4	Ν	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	4	L1	JMP	Y	3.1	Ν	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	5	L1	JMP	Ν	<1	Ν	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	6	L1	JMP	Y	144.5	Y	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	7	L1	JMP	Ν	<1	Ν	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	8	L1	JMP	Ν	<1	Y	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	9	L1	JMP	Ν	<1	Ν	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	10	L1	JMP	Ν	<1	Ν	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	11	L1	JMP	Y	2.0	Y	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	12	L1	JMP	Y	6.4	Ν	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	13	L1	JMP	Y	2.0	Ν	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	14	L1	JMP	Y	1.0	Ν	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	15	L1	JMP	Y	>200.5	Y	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	16	L1	JMP	Ν	<1	Ν	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	17	L1	JMP	Ν	<1	Ν	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	18	L1	JMP	Ν	<1	Ν	0.0
Roxas City	2-Mar-10	3-Mar-10	B5	Cabugao	19	L1	DWP	Ν	<1	Ν	0.0
Mambusao	22-Jan-10	23-Jan-10	B1	Caidquid	1	D	US	N	<1	N	0.0
Mambusao	22-Jan-10	23-Jan-10	B1	Caidquid	2	L1	JMP	Y	88.5	Y	1.0

Municipality	Sampling Date	Reading Date	Barangay Number	<i>Barangay</i> Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Mambusao	22-Jan-10	23-Jan-10	B1	Caidquid	3	D	OD	Y	12.4	Y	0.0
Mambusao	22-Jan-10	23-Jan-10	B1	Caidquid	4	L1	JMP	N	<1	N	0.0
Mambusao	22-Jan-10	23-Jan-10	B1	Caidquid	5	L1	JMP	N	<1	N	0.0
Mambusao	22-Jan-10	23-Jan-10	B1	Caidquid	6	L1	JMP	N	<1	Y	0.0
Mambusao	22-Jan-10	23-Jan-10	B1	Caidquid	7	D	OD	Y	2.0	N	0.0
Mambusao	22-Jan-10	23-Jan-10	B1	Caidquid	8	D	US	N	<1	N	0.0
Mambusao	22-Jan-10	23-Jan-10	B1	Caidquid	9	L1	JMP	Y	16.4	Y	1.0
Mambusao	22-Jan-10	23-Jan-10	B1	Caidquid	10	D	US	Y	2.0	Y	0.0
Mambusao	21-Dec-09	22-Dec-09	B2	Batoz	1	L1	JMP	N	<1	N	0.0
Mambusao	21-Dec-09	22-Dec-09	B2	Batoz	2	L1	JMP	Y	3.1	N	0.0
Mambusao	21-Dec-09	22-Dec-09	B2	Batoz	3	L1	JMP	Y	32.4	Y	0.0
Mambusao	21-Dec-09	22-Dec-09	B2	Batoz	4	L1	JMP	Y	>200.5	Y	5.0
Mambusao	21-Dec-09	22-Dec-09	B2	Batoz	5	L1	JMP	N	<1	N	0.0
Mambusao	21-Dec-09	22-Dec-09	B2	Batoz	6	L1	JMP	Y	2.0	Y	0.0

Municipality	Sampling Date	Reading Date	Barangay Number	<i>Barangay</i> Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Mambusao	21-Dec-09	22-Dec-09	B9	Tumalahid	7	L1	JMP	Y	>200.5	Y	1.0
Mambusao	21-Dec-09	22-Dec-09	B9	Tumalahid	8	L1	JMP	Y	6.4	Y	1.0
Mambusao	21-Dec-09	22-Dec-09	B9	Tumalahid	9	L1	JMP	Y	>200.5	Y	12.0
Mambusao	21-Dec-09	22-Dec-09	B9	Tumalahid	10	L1	JMP	Y	11.1	Y	0.0
Mambusao	21-Dec-09	22-Dec-09	B9	Tumalahid	11	L1	JMP	Y	>200.5	Y	6.0
Sapian	6-Jan-10	7-Jan-10	B1	Bilao	11	L1	JMP	Y	1.0	Ν	0.0
Sapian	6-Jan-10	7-Jan-10	B1	Bilao	12	L1	JMP	Ν	<1	Ν	0.0
Sapian	6-Jan-10	7-Jan-10	B1	Bilao	15	L1	JMP	Y	2.0	Y	1.0
Sapian	6-Jan-10	7-Jan-10	B1	Bilao	13	L1	JMP	Y	32.4	Y	1.0
Sapian	6-Jan-10	7-Jan-10	B1	Bilao	14	L1	JMP	Y	78.2	Y	0.0
Sapian	6-Jan-10	7-Jan-10	B2	Lonoy	16	L1	JMP	Y	>200.5	Y	62.0
Sapian	6-Jan-10	7-Jan-10	B2	Lonoy	17	L1	PS	Y	>200.5	Y	7.0
Sapian	6-Jan-10	7-Jan-10	B2	Lonoy	18	L1	DWP	Y	83.1	Y	2.0
Sapian	6-Jan-10	7-Jan-10	B2	Lonoy	19	L1	JMP	Ν	<1	Ν	0.0
Sapian	6-Jan-10	7-Jan-10	B2	Lonoy	20	L1	JMP	Y	>200.5	Y	22.0
Sapian	21-Dec-09	22-Dec-09	B3	Maninang	1	L1	SWP	Y	36.4	Y	1.0
Sapian	21-Dec-09	22-Dec-09	B3	Maninang	2	L1	SWP	Y	40.6	Y	4.0
Sapian	21-Dec-09	22-Dec-09	B3	Maninang	3	L1	SWP	Ν	<1	Ν	0.0
Sapian	21-Dec-09	22-Dec-09	B3	Maninang	4	L1	SWP	Y	50.4	Y	2.0
Sapian	21-Dec-09	22-Dec-09	B3	Maninang	5	L1	PDW	Y	>200.5	Y	2.0
Sapian	21-Dec-09	22-Dec-09	B4	Poblacion	6	L1	DWP	Y	>200.5	Y	34.0
Sapian	21-Dec-09	22-Dec-09	B4	Poblacion	7	L1	SWP	Y	28.8	Y	1.0
Sapian	21-Dec-09	22-Dec-09	B4	Poblacion	8	L1	SWP	Y	1.0	Y	0.0

Municipality	Sampling Date	Reading Date	Barangay Number	Barangay Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Sapian	21-Dec-09	22-Dec-09	B4	Poblacion	9	L1	SWP	Y	5.3	Ν	0.0
Sapian	21-Dec-09	22-Dec-09	B4	Poblacion	10	L1	SWP	Y	>200.5	Y	6.0
Sigma	21-Dec-09	22-Dec-09	B1	Parian	1	L1	JMP	Y	15.0	Y	2.0
Sigma	21-Dec-09	22-Dec-09	B1	Parian	2	L1	JMP	Y	34.4	Y	0.0
Sigma	21-Dec-09	22-Dec-09	B1	Parian	3	D	OD	Y	>200.5	Ν	14.0
Sigma	21-Dec-09	22-Dec-09	B1	Parian	4	L1	JMP	Y	>200.5	Y	15.0
Sigma	21-Dec-09	22-Dec-09	B2	Malapad Pogon	5	L1	SWP	Ν	<1	Ν	0.0
Sigma	21-Dec-09	22-Dec-09	B2	Malapad Pogon	6	L1	JMP	Ν	<1	Y	0.0
Sigma	21-Dec-09	22-Dec-09	B2	Malapad Pogon	7	L1	JMP	Y	118.4	Y	6.0
Sigma	21-Dec-09	22-Dec-09	B3	Amaga	8	L1	DWP	Ν	<1	Y	0.0
Sigma	21-Dec-09	22-Dec-09	B3	Amaga	9	L1	JMP	Y	144.5	Y	1.0
Sigma	26-Jan-10	27-Jan-10	B4	Acbo	1	L2	DWP	Y	4.2	Y	0.0
Sigma	26-Jan-10	27-Jan-10	B4	Acbo	2	L2	DWP	Ν	<1	Y	0.0
Sigma	26-Jan-10	27-Jan-10	B4	Acbo	3	L2	DWP	Y	3.1	Ν	0.0
Sigma	26-Jan-10	27-Jan-10	B4	Acbo	4	L2	DWP	Y	129.8	Y	6.0
Sigma	26-Jan-10	27-Jan-10	B4	Acbo	5	L2	DWP	Y	7.5	Ν	0.0
Sigma	23-Feb-10	24-Feb-10	B5	Poblacion Norte	1	L3	LWUA	Ν	<1	N	0.0
Sigma	23-Feb-10	24-Feb-10	B5	Poblacion Norte	2	L3	LWUA	Ν	<1	Ν	0.0
Sigma	23-Feb-10	24-Feb-10	B5	Poblacion Norte	3	L3	LWUA	Y	2.0	Ν	0.0
Sigma	23-Feb-10	24-Feb-10	B5	Poblacion Norte	4	L3	LWUA	Ν	<1	Ν	0.0
Sigma	23-Feb-10	24-Feb-10	B5	Poblacion Norte	5	L3	LWUA	Ν	<1	Ν	0.0
Sigma	23-Feb-10	24-Feb-10	B6	Poblacion Sur	6	L3	LWUA	N	<1	Y	0.0
Sigma	23-Feb-10	24-Feb-10	B6	Poblacion Sur	7	L3	LWUA	Ν	<1	Ν	0.0
Sigma	23-Feb-10	24-Feb-10	B6	Poblacion Sur	8	L3	LWUA	N	<1	N	0.0
Sigma	23-Feb-10	24-Feb-10	B6	Poblacion Sur	9	L3	LWUA	Ν	<1	N	0.0
Sigma	23-Feb-10	24-Feb-10	B6	Poblacion Sur	10	L3	LWUA	Ν	<1	Ν	0.0
Sigma	9-Mar-10	10-Mar-10	B7	Matangcong	1	L1	PDW	Y	1.0	Ν	0.0

Municipality	Sampling Date	Reading Date	Barangay Number	<i>Barangay</i> Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Sigma	9-Mar-10	10-Mar-10	B8	Dayhagon	2	D	OD	Y	>200.5	Y	3.0
Sigma	9-Mar-10	10-Mar-10	B8	Dayhagon	3	D	OD	Y	>200.5	Y	12.0
Sigma	9-Mar-10	10-Mar-10	B8	Dayhagon	4	D	OD	Ν	<1	Ν	1.0
Sigma	9-Mar-10	10-Mar-10	B8	Dayhagon	5	D	OD	Y	>200.5	Y	0.0
Sigma	9-Mar-10	10-Mar-10	B9	Pagbunitan	6	L1	RW	Y	>200.5	Y	6.0
Sigma	9-Mar-10	10-Mar-10	B9	Pagbunitan	7	L1	RW	Ν	<1	Y	5.0
Sigma	9-Mar-10	10-Mar-10	B9	Pagbunitan	8	L1	RW	Ν	<1	Ν	10.0
Sigma	9-Mar-10	10-Mar-10	B9	Pagbunitan	9	L1	RW	Ν	<1	Ν	9.0
Sigma	9-Mar-10	10-Mar-10	B9	Pagbunitan	10	L1	RW	Y	1.0	Ν	4.0
Sigma	9-Mar-10	10-Mar-10	B9	Pagbunitan	11	L1	RW	Ν	<1	Ν	8.0
Jamindan	21-Dec-09	22-Dec-09	B3	Esperanza	1	L1	PS	Y	9.9	Y	0.0
Jamindan	21-Dec-09	22-Dec-09	B3	Esperanza	2	L1	PS	Y	>200.5	Ν	0.0
Jamindan	21-Dec-09	22-Dec-09	B3	Esperanza	4	L1	SWP	Ν	<1	Y	15.0
Jamindan	21-Dec-09	22-Dec-09	B3	Esperanza	5	L1	JMP	Y	>200.5	Y	2.0
Jamindan	21-Dec-09	22-Dec-09	B4	Jagnaya	1	L1	JMP	Y	>200.5	Y	TNTC
Jamindan	21-Dec-09	22-Dec-09	B4	Jagnaya	3	L1	RW	Y	>200.5	Y	36.0
Jamindan	21-Dec-09	22-Dec-09	B4	Jagnaya	4	L1	RW	Ν	<1	Y	0.0
Jamindan	21-Dec-09	22-Dec-09	B4	Jagnaya	5	L1	JMP	Ν	<1	Ν	0.0
Jamindan	21-Dec-09	22-Dec-09	B6	Pangabuan	2	L1	JMP	Ν	<1	Y	0.0
Jamindan	21-Dec-09	22-Dec-09	B6	Pangabuan	3	L1	JMP	Y	47.8	Ν	0.0
Jamindan	21-Dec-09	22-Dec-09	B6	Pangabuan	5	L1	JMP	Y	>200.5	Y	38.0
Jamindan	26-Jan-10	27-Jan-10	B14	Jaena Norte	1	L1	DWP	Y	>200.5	Y	0.0
Jamindan	26-Jan-10	27-Jan-10	B14	Jaena Norte	2	L1	JMP	Y	165.2	Ν	0.0
Jamindan	26-Jan-10	27-Jan-10	B14	Jaena Norte	3	L1	JMP	Y	200.5	Y	0.0
Jamindan	26-Jan-10	27-Jan-10	B14	Jaena Norte	4	L1	JMP	Y	200.5	Ν	0.0
Jamindan	26-Jan-10	27-Jan-10	B14	Jaena Norte	5	L1	JMP	Y	200.5	Y	0.0
Jamindan	26-Jan-10	27-Jan-10	B6sub2	Baye Baye	1	L1	JMP	Y	59.7	Y	3.0

Municipality	Sampling Date	Reading Date	Barangay Number	Barangay Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Jamindan	26-Jan-10	27-Jan-10	B6sub2	Baye Baye	2	L1	JMP	Y	1.0	Y	2.0
Jamindan	26-Jan-10	27-Jan-10	B6sub2	Baye Baye	3	L1	JMP	Y	3.1	Y	21.0
Jamindan	26-Jan-10	27-Jan-10	B6sub2	Baye Baye	4	L1	JMP	Y	5.3	Y	2.0
Jamindan	26-Jan-10	27-Jan-10	B6sub2	Baye Baye	5	L1	JMP	Y	2.0	Y	3.0
Jamindan	27-Jan-10	28-Jan-10	B1	Agambulong	1	D	OD	Y	>200.5	Y	1.0
Jamindan	27-Jan-10	28-Jan-10	B1	Agambulong	2	D	OD	Y	>200.5	Y	2.0
Jamindan	27-Jan-10	28-Jan-10	B1	Agambulong	3	D	OD	Y	>200.5	Y	2.0
Jamindan	27-Jan-10	28-Jan-10	B1	Agambulong	4	D	OD	Y	>200.5	Y	63.0
Jamindan	27-Jan-10	28-Jan-10	B1	Agambulong	5	D	OD	Y	>200.5	Y	8.0
Jamindan	23-Feb-10	24-Feb-10	B26	Poblacion	1	L2	GPS	Ν	<1	Y	0.0
Jamindan	23-Feb-10	24-Feb-10	B26	Poblacion	2	L2	GPS	Ν	<1	Y	0.0
Jamindan	23-Feb-10	24-Feb-10	B26	Poblacion	3	L2	GPS	Y	73.8	Y	2.0
Jamindan	23-Feb-10	24-Feb-10	B26	Poblacion	4	L2	GPS	Ν	<1	Y	0.0
Jamindan	23-Feb-10	24-Feb-10	B26	Poblacion	5	L2	GPS	Y	1.0	Y	0.0
Panitan	2-Mar-10	3-Mar-10	B2	Balatucan	1	L1	JMP	Ν	<1	Ν	0.0
Panitan	2-Mar-10	3-Mar-10	B2	Balatucan	2	L1	JMP	Y	1.0	Ν	0.0
Panitan	2-Mar-10	3-Mar-10	B2	Balatucan	3	L1	JMP	Ν	<1	Ν	0.0
Panitan	2-Mar-10	3-Mar-10	B2	Balatucan	4	L1	JMP	Y	1.0	Ν	0.0
Panitan	2-Mar-10	3-Mar-10	B2	Balatucan	5	L1	JMP	Y	>200.5	Y	2.0
Panitan	2-Mar-10	3-Mar-10	B3	Timpas	1	L1	JMP	Y	>200.5	Y	5.0
Panitan	2-Mar-10	3-Mar-10	B3	Timpas	2	L1	JMP	Y	4.2	Y	0.0
Panitan	2-Mar-10	3-Mar-10	B3	Timpas	3	L1	JMP	Y	3.1	Y	0.0
Panitan	2-Mar-10	3-Mar-10	B3	Timpas	4	L1	JMP	Ν	<1	Ν	0.0
Panitan	2-Mar-10	3-Mar-10	B3	Timpas	5	L1	JMP	Ν	<1	Ν	0.0
Panitan	25-Jan-10	26-Jan-10	B1	Capagao	1	L1	JMP	Y	64.5	Y	0.0
Panitan	25-Jan-10	26-Jan-10	B1	Capagao	2	L1	JMP	Y	1.0	N	0.0
Panitan	25-Jan-10	26-Jan-10	B1	Capagao	3	L1	JMP	Y	1.0	Ν	0.0

Municipality	Sampling Date	Reading Date	Barangay Number	Barangay Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
Panitan	25-Jan-10	26-Jan-10	B1	Capagao	4	L1	JMP	Ν	<1	Ν	0.0
Panitan	25-Jan-10	26-Jan-10	B1	Capagao	5	L1	JMP	Ν	<1	Ν	0.0
Panay	25-Jan-10	26-Jan-10	B4	Magubilan	1	D	OD	Y	>200.5	Y	4.0
Panay	25-Jan-10	26-Jan-10	B4	Magubilan	2	D	OD	Y	>200.5	Y	2.0
Panay	25-Jan-10	26-Jan-10	B4	Magubilan	3	D	OD	Y	53.1	Y	7.0
Panay	25-Jan-10	26-Jan-10	B4	Magubilan	4	D	OD	Y	>200.5	Y	6.0
Panay	25-Jan-10	26-Jan-10	B4	Magubilan	5	D	OD	Y	28.8	Y	0.0
Panay	16-Mar-10	17-Mar-10	B6	Bahit	1	L1	JMP	Ν	<1	Ν	0.0
Panay	16-Mar-10	17-Mar-10	B6	Bahit	2	L1	JMP	Ν	<1	Ν	0.0
Panay	16-Mar-10	17-Mar-10	B6	Bahit	3	L1	JMP	Y	15.0	Y	0.0
Panay	16-Mar-10	17-Mar-10	B6	Bahit	4	L1	JMP	Y	16.4	Y	0.0
Panay	16-Mar-10	17-Mar-10	B6	Bahit	5	L1	JMP	Y	>200.5	Y	1.0
Panay	16-Mar-10	17-Mar-10	B6	Bahit	6	L1	JMP	Y	1.0	Ν	0.0
Panay	16-Mar-10	17-Mar-10	B5	Binangig	7	L1	JMP	Y	36.4	Ν	1.0
Panay	16-Mar-10	17-Mar-10	B5	Binangig	8	L1	JMP	Y	1.0	Ν	0.0
Panay	16-Mar-10	17-Mar-10	B5	Binangig	9	L1	JMP	Y	1.0	Y	0.0
Panay	16-Mar-10	17-Mar-10	B5	Binangig	10	L1	JMP	Y	28.8	Y	1.0
Panay	16-Mar-10	17-Mar-10	B5	Binangig	11	L1	JMP	Y	3.1	Ν	0.0
Panay	16-Mar-10	17-Mar-10	B5	Binangig	12	L1	JMP	Y	>200.5	Y	5.0
Panay	16-Mar-10	17-Mar-10	B5	Binangig	13	L1	JMP	Ν	9.9	Y	0.0
Panay	16-Mar-10	17-Mar-10	B5	Binangig	14	L1	JMP	Y	<1	Ν	0.0
Panay	16-Mar-10	17-Mar-10	B5	Binangig	15	L1	JMP	Y	6.4	Ν	0.0
Panay	16-Mar-10	17-Mar-10	B5	Binangig	16	L1	JMP	Y	1.0	Ν	0.0
President Roxas	7-Jan-10	8-Jan-10	B1	Poblacion	1	D	OD	Y	9.9	Y	0
President Roxas	7-Jan-10	8-Jan-10	B1	Poblacion	2	D	OD	Y	> 200.5	Y	17

Municipality	Sampling Date	Reading Date	Barangay Number	Barangay Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
President Roxas	7-Jan-10	8-Jan-10	B1	Poblacion	3	D	OD	Y	> 200.5	Y	4
President Roxas	7-Jan-10	8-Jan-10	B1	Poblacion	4	L1	PDW	Y	> 200.5	Y	20
President Roxas	7-Jan-10	8-Jan-10	B1	Poblacion	5	D	OD	Y	> 200.5	Y	2
President Roxas	8-Jan-10	9-Jan-10	B13	Cubay	1	L1	PS	Y	> 200.5	Y	4
President Roxas	8-Jan-10	9-Jan-10	B13	Cubay	2	L1	PS	Y	5.3	Y	0
President Roxas	7-Jan-10	8-Jan-10	B3	Hanglid	1	L1	PDW	Y	> 200.5	Y	15
President Roxas	7-Jan-10	8-Jan-10	B3	Hanglid	2	D	OD	Y	200.5	Y	20
President Roxas	7-Jan-10	8-Jan-10	B3	Hanglid	3	L1	PDW	Y	9.9	N	0
President Roxas	10-Dec-09	11-Dec-09	B11	Sangkal	1	L1	RW	Y	7.5	N	0
President Roxas	10-Dec-09	11-Dec-09	B11	Sangkal	2	L1	RW	N	<1	N	0
President Roxas	10-Dec-09	11-Dec-09	B11	Sangkal	3	L1	RW	Y	1	Y	0
President Roxas	10-Dec-09	11-Dec-09	B11	Sangkal	4	L1	RW	Y	20.7	N	0
President Roxas	10-Dec-09	11-Dec-09	B11	Sangkal	5	L1	RW	Y	1	N	0
President Roxas	10-Dec-09	11-Dec-09	B11	Sangkal	6	L1	RW	Y	3.1	Y	0

Municipality	Sampling Date	Reading Date	Barangay Number	Barangay Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
President Roxas	10-Dec-09	11-Dec-09	B11	Sangkal	7	L1	RW	Y	5.3	Y	0
President Roxas	10-Dec-09	11-Dec-09	B19	Viscaya	1	L1	JMP	N	<1	N	0
President Roxas	10-Dec-09	11-Dec-09	B19	Viscaya	2	L1	JMP	N	<1	N	0
President Roxas	10-Dec-09	11-Dec-09	B19	Viscaya	3	L1	JMP	Y	3.1	N	0
President Roxas	4-Feb-10	5-Feb-10	B20	Badiangon	1	L2	GPS	Y	5.3	N	0
President Roxas	4-Feb-10	5-Feb-10	B20	Badiangon	2	L2	GPS	Y	1	N	0
President Roxas	4-Feb-10	5-Feb-10	B20	Badiangon	3	L2	GPS	N	<1	N	0
President Roxas	4-Feb-10	5-Feb-10	B20	Badiangon	4	L2	GPS	Y	1	N	0
President Roxas	4-Feb-10	5-Feb-10	B20	Badiangon	5	L2	GPS	Y	1	N	0
President Roxas	18-Feb-10	19-Feb-10	B4	Pantalan	1	L3	LWUA	N	<1.0	N	0
President Roxas	18-Feb-10	19-Feb-10	B4	Pantalan	2	L3	LWUA	N	<1.0	N	0
President Roxas	18-Feb-10	19-Feb-10	B4	Pantalan	3	L3	LWUA	N	<1.0	N	0
President Roxas	18-Feb-10	19-Feb-10	B4	Pantalan	4	L3	LWUA	N	<1.0	N	0
President Roxas	18-Feb-10	19-Feb-10	B4	Pantalan	5	L3	LWUA	N	<1.0	N	0

Municipality	Sampling Date	Reading Date	Barangay Number	<i>Barangay</i> Name	Sample Number	Water Source Level	Water Source Type	Quanti-Tray Fluorescence	Quanti- Tray <i>E.coli</i> MPN	Colilert Fluorescence	Petrifilm(blue colonies with gas bubbles)
President Roxas	18-Feb-10	19-Feb-10	B1	Poblacion Campo	6	L3	LWUA	N	<1.0	N	0
President Roxas	18-Feb-10	19-Feb-10	B1	Poblacion Campo	7	L3	LWUA	N	<1.0	N	0
President Roxas	18-Feb-10	19-Feb-10	B1	Poblacion Campo	8	L3	LWUA	N	<1.0	N	0
President Roxas	18-Feb-10	19-Feb-10	B1	Poblacion Campo	9	L3	LWUA	N	<1.0	N	0
President Roxas	18-Feb-10	19-Feb-10	B1	Poblacion Campo	10	L3	LWUA	N	<1.0	N	0

Appendix C: Charles River Water Quality Results

					Quant-Tray®/2	2000 Result	EC-Kit l	Result
Collection Date	Dilution	NO	L#	TYPE OF SOURCE	FLUORESCE? (Y/N)	MPN/100 ml E.COLI	FLUORESCE? (Y/N)	NO. OF BLUE COLONIES w/ GAS BUBBLES
4-Apr-10	1/100	1	D	CRW	Y	9.8	Y	0
4-Apr-10	1/100	2	D	CRW	Y	12.1	Y	0
4-Apr-10	1/100	3	D	CRW	Y	10.8	Y	0
4-Apr-10	1/100	4	D	CRW	Y	5.2	Y	0
4-Apr-10	2/100	1	D	CRW	Y	17.1	Y	0
4-Apr-10	2/100	2	D	CRW	Y	27.9	Y	0
4-Apr-10	2/100	3	D	CRW	Y	32.7	Y	0
4-Apr-10	2/100	4	D	CRW	Y	28.5	Y	0
4-Apr-10	5/100	1	D	CRW	Y	60.2	Y	0
4-Apr-10	5/100	2	D	CRW	Y	74.9	Y	0
4-Apr-10	5/100	3	D	CRW	Y	54.6	Y	0
4-Apr-10	5/100	4	D	CRW	Y	72.3	Y	0
4-Apr-10	10/100	1	D	CRW	Y	111.2	Y	1
4-Apr-10	10/100	2	D	CRW	Y	104.6	Y	1
4-Apr-10	10/100	3	D	CRW	Y	110.0	Y	0
4-Apr-10	10/100	4	D	CRW	Y	118.7	Y	2
4-Apr-10	15/100	1	D	CRW	Y	231.0	Y	0
4-Apr-10	15/100	2	D	CRW	Y	178.2	Y	1
4-Apr-10	15/100	3	D	CRW	Y	178.5	Y	1
4-Apr-10	15/100	4	D	CRW	Y	235.9	Y	0
4-Apr-10	25/100	1	D	CRW	Y	307.6	Y	1
4-Apr-10	25/100	2	D	CRW	Y	387.3	Y	2

					Quant-Tray®/2	2000 Result	EC-Kit Result		
Collection Date	Dilution	NO	L#	TYPE OF SOURCE	FLUORESCE? (Y/N)	MPN/100 ml E.COLI	FLUORESCE? (Y/N)	NO. OF BLUE COLONIES w/ GAS BUBBLES	
4-Apr-10	25/100	3	D	CRW	Y	387.3	Y	1	
4-Apr-10	25/100	4	D	CRW	Y	461.1	Y	1	
4-Apr-10	50/100	1	D	CRW	Y	387.3	Y	1	
4-Apr-10	50/100 Dup	1	D	CRW	Y	488.4	Y	1	
4-Apr-10	50/100	2	D	CRW	Y	613.1	Y	2	
4-Apr-10	50/100	3	D	CRW	Y	648.8	Y	0	
4-Apr-10	50/100 Dup	3	D	CRW	Y	980.4	Y	3	
4-Apr-10	50/100	4	D	CRW	Y	613.1	Y	1	
4-Apr-10	75/100	1	D	CRW	Y	686.7	Y	1	
4-Apr-10	75/100	2	D	CRW	Y	648.8	Y	0	
4-Apr-10	75/100	3	D	CRW	Y	727.0	Y	1	
4-Apr-10	75/100	4	D	CRW	Y	613.1	Y	4	
4-Apr-10	100/100	1	D	CRW	Y	1413.6	Y	3	
4-Apr-10	100/100	2	D	CRW	Y	1553.1	Y	5	
4-Apr-10	100/100	3	D	CRW	Y	1732.9	Y	3	
4-Apr-10	100/100	4	D	CRW	Y	1299.7	Y	5	
4-Apr-10	Blank	1	D	CRW	Ν	<1	Ν	0	
4-Apr-10	Blank	2	D	CRW	Ν	<1	Ν	0	

Appendix D: GPS Coordinates

Date of Sampling	Municipality	Barangay Name	Barangay Number	Latitude	Longitude
7-Jan-10	Pontevedra	Ameligan	B3	11°29.625'	122°50.858'
7-Jan-10	Pontevedra	Ameligan	B3	11°29.626'	122°50.757'
7-Jan-10	Pontevedra	Nelia Manaay	B23	11°28.692'	122°49.051'
7-Jan-10	Pontevedra	Nelia Manaay	B23	11°28.404'	122°48.716'
7-Jan-10	Pontevedra	Guba	B10	11°29.173'	122°48.957'
7-Jan-10	Pontevedra	Guba	B10	11°29.184'	122°48.939'
7-Jan-10	Pontevedra	Guba	B10	11°29.163'	122°48.906'
7-Jan-10	Pontevedra	Guba	B10	11°29.076'	122°48.994'
7-Jan-10	Pontevedra	Tacas	B25	11°28.391'	122°50.083'
7-Jan-10	Pontevedra	Tacas	B25	11°26.132'	122°50.334'
7-Jan-10	President Roxas	Hanglid	B3	11°25.841'	122°55.396'
7-Jan-10	President Roxas	Hanglid	B3	11°25.804'	122°55.385'
7-Jan-10	President Roxas	Hanglid	B3	11°25.788'	122°55.431'
7-Jan-10	President Roxas	Poblacion	B10	11°26.014'	122°55.523'
7-Jan-10	President Roxas	Poblacion	B10	11°25.989'	122°55.678'
7-Jan-10	President Roxas	Poblacion	B10	11°25.792'	122°55.839'
7-Jan-10	President Roxas	Poblacion	B10	11°25.767'	122°55.721'
7-Jan-10	President Roxas	Poblacion	B10	11°25.628'	122°55.544'
8-Jan-10	Maayon	Ilawod	B5	11°23.118'	122°46.085'
8-Jan-10	Maayon	Ilawod	B5	11°23.130'	122°46.118'
8-Jan-10	Maayon	Ilawod	B5	11°23.118'	122°46.085'
8-Jan-10	Maayon	Poblacion Tabuc	B6	11°23.436'	122°46.980'
8-Jan-10	Maayon	Poblacion Tabuc	B6	11°23.440'	122°46.976'
8-Jan-10	Maayon	Palaguian	B4	11°24.238'	122°46.751'
8-Jan-10	Maayon	Quinat-Uyan	B1	11°23.084'	122°47.343'
8-Jan-10	Maayon	Batabat	B2	11°22.215'	122°47.984'
8-Jan-10	President Roxas	Cubay	B13	11°25.368'	122°57.866'
8-Jan-10	President Roxas	Cubay	B13	11°25.405'	122°57.817'
8-Jan-10	Pilar	San Pedro	B4	11°29.942'	123°03.995'
8-Jan-10	Pilar	San Pedro	B4	11°29.933'	123°03.936'
8-Jan-10	Pilar	San Pedro	B4	11°29.903'	123°03.956'
8-Jan-10	Pilar	San Pedro	B4	11°30.163'	123°03.769'
8-Jan-10	Pilar	San Pedro	B4	11°29.550'	123°03.892'
12-Jan-10	Dumalag	San Roque	BSR	11°18.556'	122°37.166'
12-Jan-10	Dumalag	San Roque	BSR	11°18.601'	122°37.151'
12-Jan-10	Dumalag	San Roque	BSR	11°18.625'	122°37.161'
12-Jan-10	Dumalag	San Roque	BSR	11°18.664'	122°37.171'

Date of Sampling	Municipality	Barangay Name	Barangay Number	Latitude	Longitude
12-Jan-10	Dumalag	San Roque	BSR	11°18.794'	122°37.154'
12-Jan-10	Dumalag	San Roque	BSR	11°18.783'	122°37.099'
12-Jan-10	Dumalag	San Roque	BSR	11°18.857'	122°37.047'
12-Jan-10	Dumalag	Santa Cruz	BSC	11°19.550'	122°38.549'
12-Jan-10	Dumalag	Santa Cruz	BSC	11°19.691'	122°38.905'
12-Jan-10	Dumalag	Santa Cruz	BSC	11°19.712'	122°39.236'
12-Jan-10	Dumalag	Santa Cruz	BSC	11°19.773'	122°38.990'
12-Jan-10	Dumalag	Santa Cruz	BSC	11°19.843'	122°39.020'
12-Jan-10	Dumalag	Santa Cruz	BSC	11°19.879'	122°39.031'
12-Jan-10	Dumalag	Santa Cruz	BSC	11°19.968'	122°39.054'
12-Jan-10	Dumalag	Santa Cruz	BSC	11°20.137'	122°39.071'
13-Jan-10	Dumalag	Concepcion	BC	11°17.947'	122°36.514'
13-Jan-10	Dumalag	Dolores	BD	11°17.771'	122°37.051'
13-Jan-10	Dumalag	Dolores	BD	11°17.429'	122°36.842'
13-Jan-10	Dumalag	Dolores	BD	11°17.224'	122°36.574'
13-Jan-10	Dumalag	Dolores	BD	11°17.079'	122°36.402'
13-Jan-10	Dumalag	Dolores	BD	11°16.905'	122°36.188'
13-Jan-10	Dumalag	Poblacion	BP	11°18.182'	122°37.220'
13-Jan-10	Dumalag	Poblacion	BP	11°18.315'	122°37.307'
13-Jan-10	Dumalag	Poblacion	BP	11°18.490'	122°37.411'
13-Jan-10	Dumalag	San Angel	SA	11°18.359'	122°39.333'
13-Jan-10	Dumalag	San Angel	SA	11°18.373'	122°39.478'
13-Jan-10	Dumalag	San Angel	SA	11°18.381'	122°39.487'
13-Jan-10	Dumalag	San Angel	SA	11°18.428'	122°39.545'
13-Jan-10	Dumalag	Poblacion	BP	11°18.438'	122°37.427'
13-Jan-10	Dumalag	Poblacion	BP	11°18.410'	122°37.426'
14-Jan-10	Ivisan	Santa Cruz	B2	11°31.054'	122°42.396'
14-Jan-10	Ivisan	Santa Cruz	B2	11°31.108'	122°42.479'
14-Jan-10	Ivisan	Santa Cruz	B2	11°31.874'	122°42.503'
14-Jan-10	Ivisan	Santa Cruz	B2	11°30.880'	122°42.631'
14-Jan-10	Ivisan	Santa Cruz	B2	11°31.077'	122°42.778'
14-Jan-10	Ivisan	Ilaya	B3	11°30.656'	122°43.058'
14-Jan-10	Ivisan	Ilaya	B3	11°30.943'	122°43.637'
14-Jan-10	Ivisan	Ilaya	B3	11°31.114	122°43.698'
14-Jan-10	Ivisan	Ilaya	B3	11°31.147'	122°43.710'
14-Jan-10	Ivisan	Ilaya	B3	11°30.999'	122°43.616'
14-Jan-10	Roxas City	Lanot	B1	11°31.012'	122°45.309'
14-Jan-10	Roxas City	Lanot	B1	11°31.015'	122°45.300'
14-Jan-10	Roxas City	Lanot	B1	11°31.281'	122°45.307'

Date of Sampling	Municipality	Barangay Name	Barangay Number	Latitude	Longitude
14-Jan-10	Roxas City	Lanot	B1	11°31.282'	122°45.334'
14-Jan-10	Roxas City	San Jose	B2	11°29.123'	122°44.202'
14-Jan-10	Roxas City	San Jose	B2	11°29.410'	122°44.468'
14-Jan-10	Roxas City	Jumaquicjic	B3	11°29.766'	122°44.788'
14-Jan-10	Roxas City	Jumaquicjic	B3	11°29.763'	122°44.766'
14-Jan-10	Roxas City	Jumaquicjic	B3	11°29.727'	122°44.909'
14-Jan-10	Roxas City	Jumaquicjic	B3	11°29.739'	122°44.972'
15-Jan-10	Ivisan	Matnog	B4	11°31.304'	122°40.371'
15-Jan-10	Ivisan	Matnog	B4	11°31.602'	122°40.620'
15-Jan-10	Ivisan	Matnog	B4	11°31.738'	122°40.422'
15-Jan-10	Ivisan	Matnog	B4	11°31.776'	122°40.401'
15-Jan-10	Ivisan	Agmalobo	B4	11°31.656'	122°40.490'
15-Jan-10	Ivisan	Agmalobo	B5	11°31.231'	122°40.641'
15-Jan-10	Ivisan	Agmalobo	B5	11°31.254'	122°40.627'
15-Jan-10	Ivisan	Agmalobo	B5	11°31.425'	122°40.797'
15-Jan-10	Ivisan	Agmalobo	B5	11°31.406'	122°40.827'
15-Jan-10	Ivisan	Agmalobo	B5	11°31.408'	122°40.862'
19-Jan-10	Dumarao	Codingle	B1	11°14.902'	122°41.727'
19-Jan-10	Dumarao	Codingle	B1	11°15.143	122°41.439'
19-Jan-10	Dumarao	Codingle	B1	11°15.205'	122°41.366'
19-Jan-10	Dumarao	Codingle	B1	11°15.264'	122°41.002'
19-Jan-10	Dumarao	Codingle	B1	11°15.337'	122°41.235'
19-Jan-10	Dumarao	Ilaya	B2	11°15.542'	122°41.080'
19-Jan-10	Dumarao	Ilaya	B2	11°15.687'	122°41.075'
19-Jan-10	Dumarao	Ilaya	B2	11°15.711'	122°41.148'
19-Jan-10	Dumarao	Ilaya	B2	11°15.687'	122°41.213'
19-Jan-10	Dumarao	Ilaya	B2	11°15.678'	122°41.316'
19-Jan-10	Dumarao	Ilawod	B3	11°15.799'	122°41.380'
19-Jan-10	Dumarao	Ilawod	B3	11°15.926'	122°41.353'
19-Jan-10	Dumarao	Ilawod	B3	11°15.947'	122°41.300'
19-Jan-10	Dumarao	Ilawod	B3	11°16.003'	122°41.280'
19-Jan-10	Dumarao	Ilawod	B3	11°15.917'	122°41.274'
20-Jan-10	Tapaz	San Julian	B6	11°17.238'	122°32.468'
20-Jan-10	Tapaz	San Julian	B6	11°17.065'	122°32.215'
20-Jan-10	Tapaz	San Julian	B6	11°16.941'	122°32.167'
20-Jan-10	Tapaz	San Julian	B6	11°17.031'	122°32.121'
20-Jan-10	Tapaz	San Julian	B6	11°17.096'	122°32.106'
20-Jan-10	Tapaz	San Nicolas	B7	11°19.907'	122°31.109'
20-Jan-10	Tapaz	San Nicolas	B7	11°19.878'	122°31.118'

Date of Sampling	Municipality	Barangay Name	Barangay Number	Latitude	Longitude
20-Jan-10	Tapaz	San Nicolas	B7	11°19.799'	122°31.153'
20-Jan-10	Tapaz	San Nicolas	B7	11°19.682'	122°31.225'
20-Jan-10	Tapaz	San Nicolas	B7	11°19.521'	122°31.338'
21-Jan-10	Dao	Matagnop	B5	11°23.445'	122°41.683'
21-Jan-10	Dao	Matagnop	B5	11°23.503'	122°41.796'
21-Jan-10	Dao	Matagnop	B5	11°23.460'	122°41.614'
21-Jan-10	Dao	Matagnop	B5	11°23.387'	122°41.360'
21-Jan-10	Dao	Matagnop	B5	11°23.625'	122°41.397'
21-Jan-10	Dao	Matagnop	B5	11°23.727'	122°41.432'
21-Jan-10	Dao	Matagnop	B5	11°23.707'	122°41.362'
21-Jan-10	Dao	Matagnop	B5	11°23.560'	122°41.351'
21-Jan-10	Dao	Matagnop	B5	11°23.487'	122°41.257'
21-Jan-10	Dao	Ilawod	B4	11°23.715'	122°41.238'
21-Jan-10	Dao	Ilawod	B4	11°23.698'	122°41.116'
21-Jan-10	Dao	Nasunogan	B6	11°22.837'	122°40.503'
21-Jan-10	Dao	Manhoy	B7	11°21.745'	122°40.060'
22-Jan-10	Mambusao	Caidquid	B1	11°24.611'	122°32.356'
22-Jan-10	Mambusao	Caidquid	B1	11°24.604'	122°32.510'
22-Jan-10	Mambusao	Caidquid	B1	11°24.855'	122°32.486'
22-Jan-10	Mambusao	Caidquid	B1	11°24.953'	122°32.269'
22-Jan-10	Mambusao	Caidquid	B1	11°25.014'	122°32.233'
22-Jan-10	Mambusao	Caidquid	B1	11°25.160'	122°32.224'
22-Jan-10	Mambusao	Caidquid	B1	11°25.319'	122°32.238'
22-Jan-10	Mambusao	Caidquid	B1	11°25.314'	122°32.347'
22-Jan-10	Mambusao	Caidquid	B1	11°25.191'	122°32.459'
22-Jan-10	Mambusao	Caidquid	B1	11°25.055'	122°32.499'
25-Jan-10	Panitan	Capagao	B1	11°26.217'	122°45.470'
25-Jan-10	Panitan	Capagao	B1	11°25.695'	122°44.547'
25-Jan-10	Panitan	Capagao	B1	11°25.946'	122°44.743'
25-Jan-10	Panitan	Capagao	B1	11°26.291'	122°44.970'
25-Jan-10	Panitan	Capagao	B1	11°25.878'	122°44.942'
25-Jan-10	Panay	Magubilan	B4	11°29.954'	122°48.040'
25-Jan-10	Panay	Magubilan	B4	11°29.779'	122°48.495'
25-Jan-10	Panay	Magubilan	B4	11°29.650'	122°48.483'
25-Jan-10	Panay	Magubilan	B4	11°29.602'	122°48.475'
25-Jan-10	Panay	Magubilan	B4	11°29.453'	122°48.510'
26-Jan-10	Sigma	Acbo	B4	11°26.734'	122°40.086'
26-Jan-10	Sigma	Acbo	B4	11°26.741'	122°40.103'
26-Jan-10	Sigma	Acbo	B4	11°26.448'	122°40.142'

Date of Sampling	Municipality	Barangay Name	Barangay Number	Latitude	Longitude
26-Jan-10	Sigma	Acbo	B4	11°26.221'	122°40.213'
26-Jan-10	Sigma	Acbo	B4	11°26.056'	122°40.379'
26-Jan-10	Sigma	Parian	B1	11°28.596'	122°41.832'
26-Jan-10	Sigma	Parian	B1	11°28.676'	122°41.884'
26-Jan-10	Sigma	Parian	B1	11°28.906'	122°41.784'
26-Jan-10	Sigma	Parian	B1	11°28.994'	122°42.062'

Appendix E: EC-Kit Instructions

Modified and Updated by Patty Chuang and Susan Murcott in May 2010

EC-Kit Instructions

Materials (provided in kit)

- Petrifilm *E.coli* / Total Coliform Plates
- WhirlPak bags
- Cooler Bag + Ice Pack
- Colilert 10 milliliter predispensed tubes
- Incubator Belt
- Cardboard + Rubberbands

- 3.5 ml sterile plastic pipette
- Blacklight + 4AA batteries
- Laminated Instructions

Setup and Quality Control Procedures

- Materials obtained locally: isopropyl (rubbing alcohol- available in pharmacies), paper towels or tissues, permanent black marker, garbage bag/masking tape or ceramic/plastic tile, soap, liquid bleach, field notebook.
- Wash hands with soap and water.
- Locate a clean, level surface. Cover surface with a large plastic garbage bag, taped down with masking tape. Or, use a square ceramic or plastic tile as a work surface. Wipe down work surface with isopropyl
- Run blanks and duplicates minimum of 5% of total samples tested using boiled, cooled water, or bottled water.
- Record all your test results in a lab notebook. Be sure to include date, each test result and observations.

Procedure for Colilert Test

- Using the black-marked 10 milliliter (mL) guide test tube provided (the one tube with colored tape in the package), mark <u>all</u> the other test tubes in your kit with a permanent black marker at the same 10 mL level.
- Label each tube with the sample name, time, and date of sample collection, initials of person sampling.
- Remove cap, without touching the inside of the cap with fingers or hand. Then fill the Colilert test tube with 10mL of sample water to the black mark 10 mL level in one of two ways.
 - Using Tap other water supply delivered via a spout or on/off spigot (e.g. hand pump, public standpipe, treatment unit spout): Fill Colilert tube to the 10 mL mark by adding water directly. Do not exceed the 10 mL black-marked level on the tube. Replace cap & invert tube several times to mix.
 - Using Sterile Plastic Bag: Collect water sample in a sterile plastic bag that has been provided in the kit, then pour directly from bag into the Colliert tube. Or, use the sterile pipette provided in kit (graduated at 1 mL) to transfer sample water from the plastic bag to the test tube 10 times. Take care not to touch the sides of

the tube or the water in the tube with the pipette. Then, replace the cap and mix the water in the test tube by inverting it several times to dissolve the nutrients.

 Put Colilert tube in top pocket of incubator belt. Tie the incubator belt around your waist and wear it non-stop for 24 hours +/- 2 hrs. This will incubate the water sample using your body heat.

Interpreting Colilert Results

After 24 hours, if samples are clear, no coliform bacteria are present (see top tube in Figure 1). If samples are slightly yellow or yellow, coliform bacteria are present (see middle and bottom tubes in Figure 1). Record as clear (absent) or yellow (present) on data sheets. If the samples fluoresce to form a milky-blue color under UV/black light, then E. coli are present (see bottom tube in Figure 2). Otherwise, if the sample does not fluoresce, then *E.coli* are not present (see top 2 tubes in Figure 2.

NOTE: 2 tubes in Figure 2 show UV/black light reflecting off the Colilert tube glass. THIS IS NOT FLUORESCING!) If *E.coli* are present, a Petrifilm test should also be performed in order to quantify (If sample risk is unknown, perform both tests).

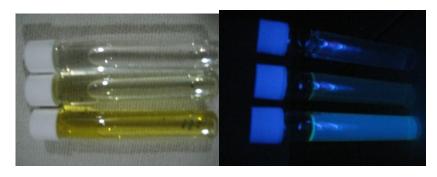


Figure 18



Procedure for Petrifilm Test

- Place the Petrifilm on a flat surface that has been wiped down with isopropyl alcohol.
- Fill sterile pipette with 1mL of sample water (1 mL= top graduated line just below top of pipette bulb)
- Lift the top film. With pipette perpendicular to Petrifilm plate, carefully dispense the 1 mL of sample from the pipette on to the center of the pink circle.
- Gently roll the top film onto the Petrifilm plate. Take care not to trap air bubbles under the top film.
- Allow the water to naturally spread out to fill the entire pink circle and allow gel to set for 1-2 minutes.
- Place the Petrifilm between two pieces of cardboard. Secure the Petrifilm between the cardboard using rubber bands.

• Place Petrifilm samples in bottom pocket of incubator belt. Up to five Petrifilms can be stacked between one set of cardboard squares. Incubate at body temperature non-stop for 24 hours +/- 2 hours at body temperature..

Interpreting Petrifilm Results

E.coli are blue colonies with gas bubbles. Total coliform are the sum of red plus blue colonies with gas bubbles. If the total number of blue colonies with gas bubbles is less then 1, then the water may still have an intermediate risk level that is below the detection limit of the Petrifilm test (See Table 1 below). If the total number of blue colonies with gas bubbles counted is between 1 and 10, this represents a high risk level. If the total number of blue colonies with gas bubbles with gas bubbles counted is above 10, this is a very high risk level.

Recommendations on Reading Colilert and Petrifilm Results

Colilert:

The UV/black light test to determine fluoresce MUST BE PERFORMED IN THE DARK (a dark room, a closet, a bathroom, or outdoors at night). Otherwise, fluoresce will not be able to be seen clearly.

Petrifilm

Must be read in bright daylight. Hold the Petrifilm up to natural light.

Must be counted SYSTEMATICALLY. (Figure 3)

Be sure to count every colony – blue with gas bubbles, red with gas bubbles, then add blue + red with gas bubbles including even very small colonies with gas bubbles.

Use the grid system on the Petrifilm plate. Begin at the top right square and proceed sequentially from square to square following the curved "S" path on the figure below. Colonies on the horizontal grid lines are "pushed down into the square below." Colonies on the vertical grid lines are pulled forward into the next square. See Figure 3.

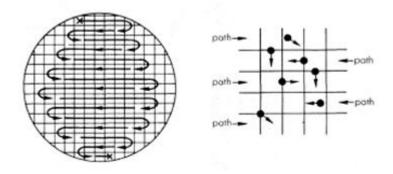


Figure 3: System for counting coliform colonies

Bubble patterns may vary. Gas may disrupt the colony so that the colony "outlines" the bubble (circles 1 and 2 in Figure 4). Artifact bubbles may result from improper inoculation or from trapped air within the sample. They are irregularly shaped and are not associated with a colony (circle 3 in Figure 4). Figure 5 shows various bubble patterns associated with gas producing colonies. All should be enumerated.

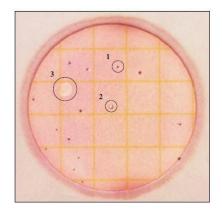


Figure 4: Sample Total Coliform Count Plate. Circles 1 and 2 are associated with colonies with gas bubbles, circle 3 is not associated with a colony.

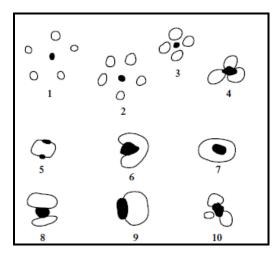


Figure 5: Various Bubble Patterns for Gas Producing Colonies.

Interpretation of EC-Kit Results for E.coli

The EC-Kit - Colilert and Petrifilm – provides results for both *E.coli* and total coliform. First, *E.coli* is discussed:

Q: Why do we care about *E.coli*?

A: E.coli is used to determine the safety of drinking water.

Q: What level of *E.coli* is safe to drink?

A: World Health Organization guideline for *E.coli*: "*E.coli* or thermotolerant coliform bacteria must not be detected in any 100 milliliter sample for all water directly intended for drinking, treated water entering the distribution system or treated water in the distribution system." (WHO, 2004)

Q: What if a water quality test shows higher *E.coli* values than the WHO guideline value of 0 *E.coli*/100 milliliter sample?

A: Refer to a risk table (see Table 1 below).

Interpretation of EC-Kit Results for E.coli using a Risk Table

The two right-hand columns of Table 1 show the World Health Organization's risk rankings for *E.coli* (WHO, 1997). At less than 1 (<1) *E.coli* colony forming units (CFU) per 100 milliliter of sample, WHO quantifies risk as "conformity" meaning that it meets the WHO Guideline value of non-detection of any *E.coli* in 100 milliliter of sample (see above). At 1-10 *E.coli* colony forming units (CFU) per 100 mL sample, WHO quantifies risk as "low," 10-100 as "intermediate," 100-1000 as "high," and greater than 1000 as "very high." Looking at the "Colilert" far left (1st) column, an "absent" result (clear, no fluorescence) is equivalent to either a

WHO risk category of "conformity" or "low" risk. A test result for Colilert that comes out "present" i.e. yellow, showing total coliform <u>and</u> showing blue fluorescence means that the Colilert tube contains at least 1 *E.coli* per 10mL of sample added. This can be equivalent to one of three risk levels, depending on the corresponding Petrifilm result. If Petrifilm counts of blue colonies <u>with gas bubbles</u> are zero, the present/yellow/fluorescent Colilert + the Petrifilm, shows intermediate risk (equivalent to WHO risk categories of between 10 – 100 colony counts /100 mL). High and very high risk waters are identified by present/yellow/blue fluorescent Colilert results and *E.coli* counts of blue colonies <u>with gas bubbles</u> on the Petrifilm test at either the 1-10 count (equivalent to WHO "high" risk level) or 10 - 100 count (equivalent to WHO "very high" risk level).

EC-Kit Results - (Metcalf, 200		Risk Level Categories (World Health Organization, 1997)		
Colilert® E. coli Result	Petrifilm TM E. coli Result	Risk Level	<i>E.coli</i> in sample (cfu/100 mL)	
Absent (no fluorescence)	0	Conformity	< 1	
Absent (no fluorescence)	0	Low	1-10	
Present (blue fluorescence)	0	Intermediate	10-100	
Present (blue fluorescence)	1-10 (blue with gas bubbles count)	High	100-1000	
Present (blue fluorescence)	> 10 (blue with gas bubbles count)	Very High	> 1000	

Table 24: Risk Levels from *E.coli*

The EC-Kit for Total Coliform

The coliform group is the most common indicator group used worldwide. The most frequently used coliform indicator tests are:

- Total Coliform
- Thermotolerant coliform (sometimes referred to as "fecal" coliform)
- E.coli

Q: Why do we care about total coliform?

A: While it is strongly desirable not to detect total coliform in drinking water, total coliform is not necessarily from the feces of humans and mammals (in contrast, *E.coli* and most thermotolerant coliform are). Some coliform can occur naturally in the environment. Therefore, *E.coli* is the most widely use test to determine whether there is human or animal feces in drinking water and total coliform is used to measure treatment performance.

Q: How is treatment performance measured?

A: Treatment performance is measured by sampling and running EC-Kit tests on at least two samples from a treatment system. The treatment system can be of any scale: large centralized treatment, community treatment or household treatment. The first sample one should collect is of the influent water – the water that supplies the treatment system. The second sample is of the treated water. By comparing the before treatment and after treatment samples, one can determine a percentage removal efficiency of the treatment system. This helps determine if the treatment system is performing effectively or not.

Interpretation of EC-Kit Results for Total Coliform

Total coliform are the <u>sum of red colonies with gas bubbles plus blue colonies with gas bubbles</u> in the Petrifilm test. Interpret the total coliform counts using Table 2.

	EC-Kit Results – Total Coliform		Total Coliform Interpretation		
	А	В	С	D	
1	Colilert® Total Coliform Result	Petrifilm TM Total Coliform Result	Combined Colilert and Petrifilm result as a total coliform count (WHO, 1997)	Standardized Unit Equivalent (for comparison, assuming a 100 milliliter sample size (which is the widely used standard)	
2	Absent (clear, no fluorescence)	0	0	<10 total coliform / 100 ml	
3	Absent (clear, no fluorescence)	0	0	<10 total coliform / 100 ml	
4	Present (yellow)	0	at least 1 total coliform per 10 ml of sample in Colilert test	At least 10 total coliform /100 ml	
5	Present (yellow)	1-10 (red with gas bubbles + blue with gas bubbles count)	1 – 10 total coliforms per 1 mL for the Petrifilm test	100-1000 total coliform/100 mL (standardized by multiplying C5 result by 100)	
6	Present (yellow)	> 10 (red with gas bubbles + blue with gas bubbles count)	10 – 100 total coliforms per 1 mL for the Petrifilm test	1000 – 10,000 total coliform/100 mL (standardized by multiplying C6 result by 100)	

Disposal of Tests

Colilert and Petrifilm tests can be safely stored for periods of days, weeks or even months, in order to be used as training tools, or to refer back to them. However, interpretation of results should only be done after 24 hours of body heat incubation.

Once you are ready to dispose of the tests, a simple, safe method is to add a few drops of household bleach (typically about 6% chlorine concentration). Add bleach to both to the Colilert tubes and to the Petfilm, by lifting the film and dispensing the drops. Allow to sit for 30 minutes, then the Colilert can be disposed down a drain, a latrine, or a dug hole. The Petrifilm can be disposed of as waste.