A Study of Alternative Microbial Indicators for Drinking Water Quality in Northern Ghana

By
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S.B. Civil and Environmental Engineering
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Submitted to the Department of Civil and Environmental Engineering and the Engineering Systems Division in partial fulfillment of the requirements for the degrees of

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ABSTRACT

Safe drinking water is essential for human survival, yet it is unavailable to over 1 billion of the world's people living in poverty (World Bank, 2009). The current methods used to identify drinking water sources are inadequate, with almost 40 percent of "safe" sources containing unsafe levels of microbial contamination (Joint Monitoring Programme (JMP) for Water Supply and Sanitation, 2010). Direct water testing is therefore necessary in order to accurately assess the safety of drinking water sources.

The goals of this thesis are as follows: (1) To confirm the accuracy of the 20ml hydrogen sulfide (H_2S) test as a single presence/absence (P/A) indicator for fecal coliforms; (2) To establish the accuracy of as a single enumerative test for fecal coliforms; (3) To verify the accuracy of the 20 ml H_2S test used in conjunction with Easygel® as an improved method of quantifying contamination as compared with the individual tests; (4) To further confirm the accuracy of the EC-Kit as an improved method of quantifying contamination as compared with the individual tests; and (5) To use the results of an informal behavioral household interviews, and a performance review of the Community Water and Sanitation Agency (CWSA) to provide context and policy recommendations to improve access to potable water in Northern Ghana.

Fieldwork for this research was completed in January 2011 in and around Tamale, Ghana. The author was hosted by Pure Home Water (PWH) and supported by the MIT Civil and Environmental Engineering Department.

Overall, the 20 ml H_2S presence/absence test was confirmed to be highly accurate for all types of water sources in Northern Ghana, as was the EC-Kit. The Easygel® and the H_2S test combination is recommended solely for use when testing improved water sources. Additionally, field observations and a review of current policies of the CWSA demonstrate significant shortcomings in the ability of the Agency to supply rural areas with safe drinking water. Recommendations for improvement include more strict regulations of the levels and nature of foreign investment in Ghana's water sector.

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List of Abbreviations

®	Registered Trademark
CFU	Colony Forming Units
CIDA	Canadian International Development Agency
CPF	Ceramic Pot Filter
CWSA	Community Water and Sanitation Agency
DA	District Assembly
DALY	Disability Adjusted Life Years
DANIDA	Danish International Development Agency
E. coli	Escherichia coli
FC	Fecal Coliform
FN	False Negative
FP	False Positive
GoG	Government of Ghana
H_2S	Hydrogen Sulfide
HWTS	Household Water Treatment and Storage
IPA	Innovations for Poverty Action
JMP	Joint Monitoring Program
KfW	German International Development Agency
M. Eng	Master of Engineering
M2	M2 Media
MDG	Millennium Development Goals
MIT	Massachusetts Institute of Technology
mL	Milliliter
MPN	Most Probably Number
MWRWH	Ministry of Water Resources, Works and Housing
NGO	Non-Governmental Organization
NTU	Nephelometric Turbidity Units
P/A	Presence/Absence
PWH	Pure Home Water
RAQWQ	Rapid Assessment of Drinking Water Quality
SODIS	Solar Disinfection
TC	Total Coliforms
™	Trademark
TR	True Result
UN	United Nations
WASH	Water, Sanitation and Hygiene
WHO	World Health Organization

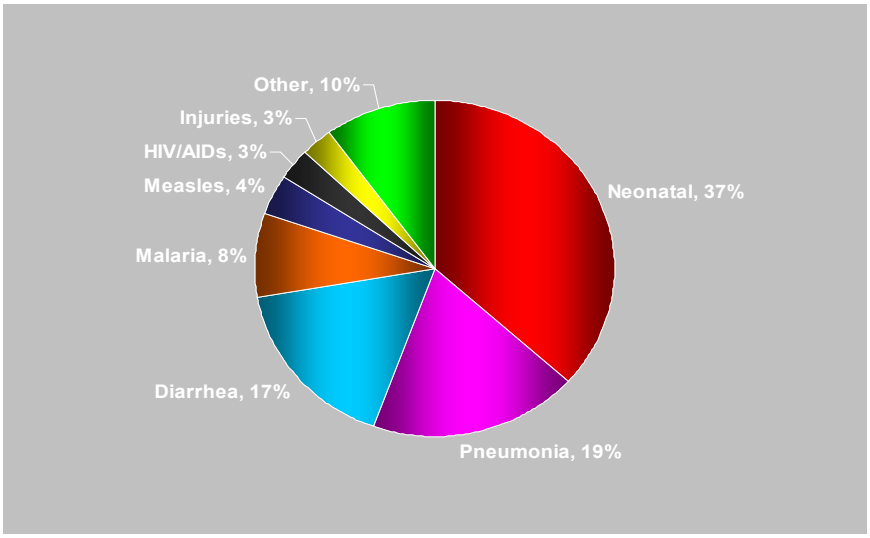
1. Motivation for Water Quality Study

1.1 Water as a Human Right

Safe drinking water is essential for human survival, yet it is unavailable to over 1 billion of the world's population living in poverty (World Bank, 2009). Almost 2 million people every year, the majority of whom are children, die from water-related diseases including diarrhea, dengue fever and typhoid, among others. Diarrhea remains in the third leading cause of death among children under five globally, killing 1.5 million children each year (World Health Organization, 2005).

In July of 2010, the UN General Assembly passed a resolution formally recognizing water and sanitation as a human right, but the world is a long way from making this right a reality (The Right to Water and Sanitation).

Figure 1. Causes of Death in Children Under Five



(World Health Organization, 2005)

In order to estimate the full impact of disease caused by pathogens transmitted via water routes, the World Bank in 1993 created an index of population health. Disability Adjusted Life Years (DALY) is the sum total of years of productive life lost due to a disability and the years of potential life lost due to premature death (World Health Organization, 2011). A disability, in this case, refers to either a physical disability or an illness resulting from the consumption of unsafe water. Though imperfect, DALYs give a better indication of the true impact these diseases have on quality of life and allow for comparison of these impacts across various diseases.

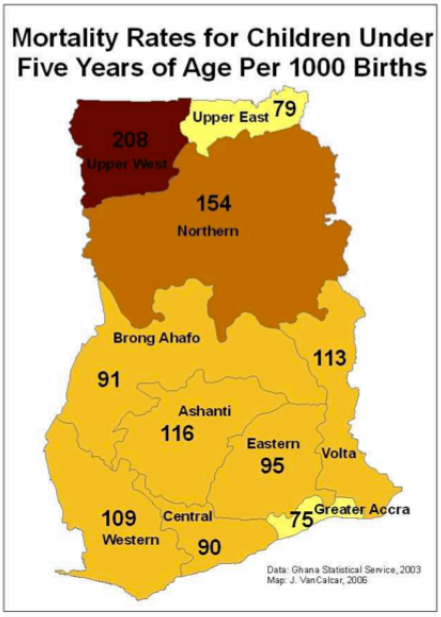
In Ghana for example, 14.6 percent of all deaths can be attributed to Water, Sanitation and Hygiene (WASH) related diseases. However, when deaths and DALYs of a widespread disease, such as diarrhea, are compared, they describe two very different scenarios. While there are 203,000 deaths in Ghana attributed to diarrhea, there are over 400,000 DALYs associated with

the disease. These statistics shows that the actual impact of the disease on societal productivity is far greater than simply the loss of life: children may be unable to attend school and adults may be kept out of work and/or unable to take care of their families (World Health Organization, 2010).

The calculated DALY for diarrhea is 19/1000 capita per year, the highest DALY found among the major diseases afflicting Northern Africa. This represents a significantly larger impact as compared other common ailments, including respiratory infections at 7.8/1000 capita, malaria at 7/1000 capita and other vector-borne diseases at 1.2/1000 capita (World Health Organization, 2009).

Figure 2 demonstrates the severity of this problem in the Northern and Upper West Regions of Ghana where the mortality rate for children under five is 154 and 208 per 1000 births, respectively. In contrast, the under-five mortality rate in the USA is 7.8 per 1000 births (World Bank, 2011).

Figure 2. Mortality Rates, Ghana



(VanCalcar, 2006)

Taken in aggregate, these statistics paint a grim picture of the impact of water quality on the quality of life in Northern Ghana.

1.2 An Introduction to Pathogens and Water Contamination

Water contamination can originate from a variety of sources, including industrial or agricultural runoff, and poorly treated, or untreated, human and animal waste. Contamination can also be naturally occurring, with chemicals, such as arsenic or fluoride, seeping into drinking water sources from geologic strata. In developing countries the most common form of contamination is microbiological[1], which comes primarily from human or animal feces mixing with drinking water sources, during transport, or at the point of use. More specifically, microbial contamination refers to the introduction of one of any number of harmful bacteria, viruses or protozoa collectively known as pathogens, into a water source.

Given the diverse nature of pathogens, it is not surprising that they behave differently when interacting with a host. While all pathogens have the ability to negatively impact the health of their host, some, such has *Legionella* and *Klebsiella,* do so only when the immune system of the host is already vulnerable, as is the often case with children, the elderly and other immune-compromised populations. Alternatively, some microbes are harmful to all members of a population, even when present at extremely low levels, as is the case with *E. coli* and *Salmonella* (World Health Organization, 1996).

The focus of this study is the detection, or indirect indication of, pathogens originating in human feces. These contaminants, referred to as enteric pathogens, are of particular concern as they more readily transmit human vectors than those originating in other warm-blooded animals.

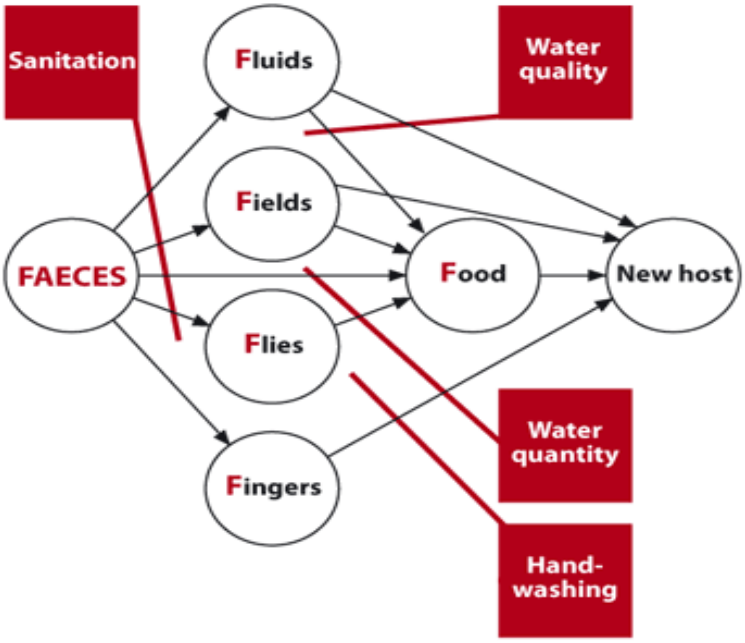
1.3 Pathogen Transmission

Figure 3, below, known as the F-Diagram, demonstrates the various ways that pathogens of fecal origin can travel to a new host. For example, open sanitation can lead to contamination via the feces-fluids route, depicted along the top of the F-Diagram. During the rainy season, surface water sources are more susceptible to contamination via runoff from areas of open defecation. If these water sources are subsequently consumed, pathogens can be transmitted to a new host.

Each of the pathways requires various aspects of WASH be improved to limit contamination. These improvements include, but are not limited to, those in the areas of sanitation, water quality, water quantity and hygiene/hand washing.

[1] The terms microbiological and microbial are used interchangeably in this thesis.

Figure 3. F-Diagram



(New Internationalist Magazine, 2011)

The World Health Organization (WHO) estimates that improvements in the water and sanitation sectors could reduce the burden of disease worldwide by 10 percent (World Health Organization, 2008). Table 1, below, provides an introduction to the classification categories associated with transmission routes and the most common examples of resulting diseases.

Table 1. Classifications of Water-Related Diseases

Classification	Transmission Details	Examples
Waterborne	Fecal-Oral Route	Cholera, Typhoid, Hepatitis A
Water-washed	Water-Hygiene	Diarrhea, Trachoma, Scabies
Water-based	Water-Contact	Guinea Worm
Insect Vector	Insect-Blood	Malaria, River Blindness

Modified from (Feachem, 1979)

1.4 United Nations (UN) Indicators of Water Quality

Millennium Development Goal (MDG) 7C is to: "Halve, by 2015, the proportion of people without sustainable access to safe drinking water and basic sanitation" (United Nations Development Program, 2010). Although awareness of a need for improvement is the water testing is known, the ability to consistently and accurately determine what is "safe" drinking water has not yet been achieved. This task must be accomplished if the global community collectively, and the developing world specifically, hopes to improve public health and raise its citizens out of poverty.

The United Nations (UN), and related organizations, currently utilize a surrogate metric for assessing drinking water quality. Rather than rely on data from water quality testing, the prevalence of "improved drinking water sources" is used to benchmark a community's drinking water quality. An improved source is determined by its ability to protect the water from outside contamination. Improved sources include a household, or community, connection to a municipal pipe, a protected dug well, rainwater or a borehole, among others, as detailed in Table 2. Unimproved sources include all unprotected surface waters and wells, tanker truck water and vended water.

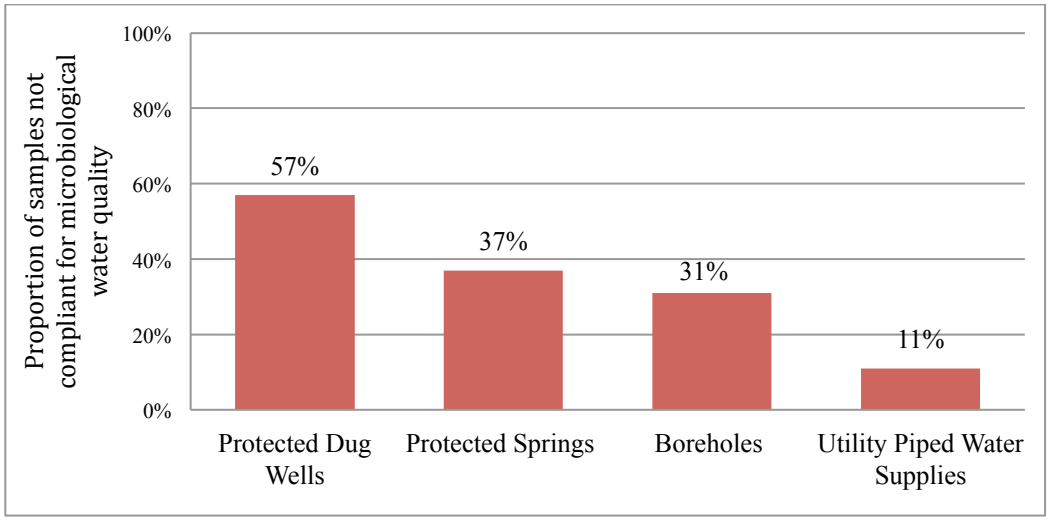
Table 2. Water Source Categories

Source Type	Examples
Unimproved	• All surface waters (rivers, streams, dams, lakes, ponds, canals) • Unprotected dug wells & springs • Tanker trucks and carts • Bottled water
Improved Piped Supply	• Household connection inside or outside user's dwelling
Other Improved	• Public taps • Tube wells & boreholes • Protected dug wells & springs • Rainwater harvesting

Improved and unimproved sources can easily be identified through a much faster and substantially cheaper process as compared with actual microbial testing. Despite these benefits, the identification of source type does not confirm the safety of a water source in a quantifiable, or necessarily reliable, manner.

The 2010 Joint Monitoring Program (JMP) Report, "Progress on Sanitation and Drinking Water," acknowledges that improved water sources can easily be contaminated, and that many improved sites, when tested, have been found to be contaminated (Joint Monitoring Programme (JMP) for Water Supply and Sanitation, 2010). The JMP followed up these observations by undertaking eight-country pilot study, the Rapid Assessment of Drinking Water Quality (RAQWQ).

Figure 4. Percent of Non-Compliant Improved Water Sources in RAQWQ Countries



(Joint Monitoring Programme (JMP) for Water Supply and Sanitation, 2010)

Figure 4, taken from the RAQWQ, shows the percentage of samples tested from various "improved" sources that did not meet microbiological standards as set by the WHO. The WHO recommended level of microbial contamination for safe drinking water is set at zero colony forming units (CFUs) per 100ml (World Health Organization, 2011). The results of the JMP report are startling with over 50 percent of protected dug wells, 37 percent of protected springs, and 31 percent of boreholes containing unsafe levels of microbial contamination. A promising statistic to highlight in this study is the fact that utility piped water sources, provided by local governments, do achieve the lowest level of microbial contamination when compared with other improved sources. Still, an average of 11 percent of piped water sources in surveyed countries[2], arguably the most trusted source of drinking water, is not safe for consumption.

Not only is there a monitoring issue in this situation with the UN reporting exaggerated progress on the Millennium Development Goals, but there is also as issue with the assumption of safety. Education programs, outreach initiatives, and publications from major international agencies publicize the safety of improved sources for consumption. This can, and has, led to increased risk for the public in developing countries, as use of the so-called "safe" sources has made them vulnerable to the host of water-related diseases resulting from microbial contamination.

The most reliable and accurate method of determining microbial water quality is by directly testing drinking water sources. While there are challenges associated with solving the problem of unsafe drinking water, a crucial first step is to obtain and disseminate information regarding the quality of existing and potential sources of water.

[2] Countries surveyed include Bangladesh, China, Ethiopia, India, Jordan, Nicaragua, Nigeria and Tajikistan.

2. Study Objectives and Area Background

2.1 Objectives

It is clear that current microbial drinking water quality monitoring methods must be improved upon to produce more accurate, lower cost, and easier-to-use tests. It is with this overarching goal in mind that the research objectives for this project were established.

1) To confirm the accuracy of the 20ml H_2S tests as a single presence/absence (P/A) indicator for fecal coliforms.
In this context, accuracy is defined as the ability of the H_2S test to detect the presence of fecal coliforms, as well as compare the results to the standard method, Quanti-Tray®, across all water source types.

2) To establish the accuracy of Easygel® as a single enumerative test for fecal coliforms.
Similar to the H_2S test*, in this context, accuracy is defined as the ability of the* Easygel® *test to enumerate the presence of fecal coliforms as well as compared the results the standard method Quanti-Tray®, across all water source types.*

3) To verify the accuracy of the 20 ml H_2S test combined with the Easygel® test as an improved method of determining the degree of microbial contamination as compared with each of the individual tests.
Results of both the 20ml H_2S test and Easygel® *tests will be correlated with a WHO Risk Level for drinking water quality. The "New Test" Risk Levels will be compared with the WHO Risk Levels indicated by the standard method Quanti-Tray® across all water source types.*

4) To further confirm the accuracy of the EC-Kit, including the 10ml pre-dispensed Colilert and 1ml Petrifilm™ test, as an improved method of quantifying contamination.
Results of both the 10ml pre-dispensed Colilert and 1ml Petrifilm™ *will be compared with the standard method, Quanti-Tray®. The pair together will then be correlated with a WHO Risk Level for drinking water quality. The "New Test" Risk Levels will be compared with the WHO Risk Levels indicated by the standard method Quanti-Tray® across all water source types.*

5) To use the results of informal behavioral household interviews and a performance review of the Community Water and Sanitation Agency (CWSA) to provide context and policy recommendations to improve access to potable water in Northern Ghana.
Drinking water quality does not exist in a black box and the quantification of contamination, though important, is not the only contributing factor in improving access to safe drinking water in the rural areas of Northern Ghana. A review of the roles, responsibilities and performance of the CWSA, as well as other minor players in the water sector, are therefore germane to include alongside microbial test recommendations.

2.2 Project Host: Pure Home Water (PHW)

Founded in 2005 by MIT Senior Lecturer Susan Murcott with local partners, PHW is a non-profit organization in Ghana whose mission is to provide safe drinking water to the people of Northern Ghana. The organization's goals are (1) To reach the people most in need of safe drinking water; and (2) To become financially and locally self-sustaining.

Earlier student teams from MIT researched the performance of, consumer preferences for, and consumer willingness to pay for, water treatment techniques in order to find the best system for the region. In addition to considering several types of ceramic filters, they investigated biosand filters, chlorination systems, and solar water disinfection (SODIS). Through these studies, PHW determined that, of the options for household water treatment and storage (HWTS) available in Northern Ghana, ceramic pot filters (CPFs) with safe storage containers, offered the simplest and cheapest method to effectively treat drinking water in Northern Ghana at the household scale.

From 2006-2011, PHW focused on distributing CPFs that were made at Ceramica Tamakloe Ltd. in Accra, Ghana, teaching people how to use them, and monitoring how effective and durable they were over time. Ceramic water filters were chosen because they were proven to be effective in removing *E. coli*, and had been shown to the reduce the number of cases of diarrhea. The filters can be manufactured almost entirely out of local materials, and are culturally appropriate since water is generally stored in large clay vessels in Northern Ghana (S. Johnson, 2008).

2.2.1 Pure Home Water Factory

As PHW grew, importing filters from Accra became less efficient. Initially, many CT filters were broken on the trip from Accra to Tamale, and over time PHW had trouble with the supplier providing pots behind schedule and of uneven quality. In order to eliminate these problems in the supply chain and better serve Northern Ghana, PHW began constructing its own factory in Tamale in January 2010. Construction of the building was still ongoing as of September 2011, however the factory has the molds, supplies, and the kiln necessary for production.

The factory currently has orders pending from NGO groups to supply filters for Northern Ghana that can be supplied once quality controls are established and quality production is ensured. The 2011-2012 MIT M.Eng Team will pursue further verification research, which must be done to ensure that filters produced at the factory consistently perform well. Research will including the monitoring of microbial removal capacity of the filters given various clay compositions and firing techniques.

2.3 Project Area

The fieldwork for this thesis was completed during the month of January 2011 in and around Tamale, Northern Ghana.

2.3.1 An Introduction to Ghana

The Republic of Ghana is located in West Africa and shares a border with neighbors Burkina Faso, Cote d'Ivoire and Togo. The country has 539 km of coastline with the Atlantic Ocean. In terms of land area, Ghana occupies 238,533 square km or is approximately the size of the U.S. state of Oregon (Central Intelligence Agency, 2011).

Figure 5. The Republic of Ghana

(Central Intelligence Agency, 2011)

Ghana gained independence from Great Britain on March 6th, 1957. From this time until the early nineties, the nation experienced a series of coups, resulting in an unstable central government for the latter half of the 20th century. In January of 1993, "The Fourth Republic," was inaugurated and has remained a stable, and relatively effective, governing body since that time (The Republic of Ghana, 2007).

The population is culturally diverse, with over 42 local dialects spoken across the country. Ghana is also divided religiously, with almost 70 percent Christian, 16 percent Muslim and 8.5 percent practicing a form of traditional tribal religions. Ghana's population is clustered between the coastal cities of Accra and Takoradi and the nation's manufacturing hub, Kumasi (The Republic of Ghana, 2007).

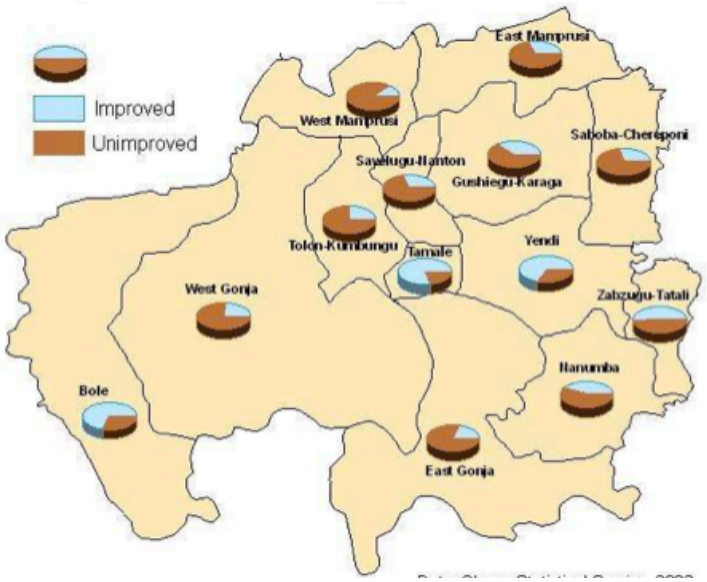
Economic prosperity varies considerably by region, with the majority of income flowing into the southern urban centers, despite over half of Ghana's population resides in rural areas. This pattern unfortunately holds for the presence of infrastructure including roads, rail systems and water supplies as well.

The focus of this study is on greater Tamale, located in the Northern Region. This area includes is among the least populated the country, representing just over 9 percent of the total population. It is also the least developed area, with 71 percent of economy based around agriculture. Only 5.7 percent of the workforce is comprised of professions with some form of higher education (Ghana Districts, 2006). Within Tamale, skilled employment and wages are substantially higher than elsewhere in the region.

2.3.2 Water Quality in Northern Ghana

With rare exception, most areas of Northern Ghana suffer from a severe lack of improved water sources, and in total, about half of the people in the region consistently rely on water from unimproved sources as shown in Figure 6. The only exception to this trend exists within Tamale itself where over 75 percent of residents are reported to have access to an improved water source. As discussed in Chapter 1, an improved source does not necessarily mean a safe source, however here it is used to gain a broad sense of the water situation in the region.

Figure 6. Improved and unimproved water sources in Northern Ghana



(VanCalcar, 2006)

2.3.3 Water Characterization of Northern Ghana

In order to gain a comprehensive understanding of the conditions in which the study results were obtained, the pH and turbidity of water samples, along with general comments on weather patterns at the time of collection, were recorded.

January is in the middle of the hot-dry season in Ghana, with precipitation almost non-existent, averaging just 0.6 inches. Temperature ranges from 75-90°F.
The average pH was determined to be 7.4 from a relatively narrow range of observed values; 7-9. This is generally considered within the normal spectrum for pH of drinking water sources.

Turbidity is a general measure for the overall water quality of a sample. Visually, turbidity is a measure of how cloudy water samples appear. Although turbid water does not necessarily indicate that water is non-potable, particles could carry potentially harmful pathogens with them. The average value, measured in Nephelometric Turbidity Units (NTU), was determined to be 124.31 NTUs. There was, however, a large observed range of turbidities, from 2 up to 1000 NTUs[3].

To provide scale, drinking water in the United States is held to standards of between 0-5 NTU, depending on the state (US Environmental Protection Agency, 2011). Harmattan, a dry, dusty

[3] Turbidity was measured in samples from both surface water sources and household drinking water containers. As many households employed a guinea worm filter prior to storage, the lower household turbidities observed are a possible result of water treatment and reflect the turbidity of the consumed water as opposed to the source.

wind off the Sahara Desert to the North, blows strongly in January, contributing to the high levels of observed turbidity. The elevated levels of turbidity, in Ghana, indicate a higher risk that water samples are microbially contaminated.

3. Indicators and Drinking Water Regulations

3.1 The Basics of Indicator Organisms

There exist two main methods to identify microbially contaminated water sources. The first is to test directly for pathogens. Direct testing means that many individual tests have to be run, as each screen tests for only one unique type of pathogen. Although a more accurate method, exhaustive testing for pathogens in drinking water can be a cumbersome process involving complicated, time consuming and often, expensive procedure, as there are a high number of pathogens that have been identified as harmful (Gerba, 2000) (Steven, 2003).

Alternatively, there is the option to use an indicator organism, or non-pathogenic bacteria, as a proxy for harmful bacteria as they are present in similar environments. Indicator organisms are determined primarily based on their presence in the human gut and their inability to exist outside of that environment for extended period of time. Thus, the presence of an indicator organism in water suggests the presence of fecal contamination and potentially, of pathogens.

The ease, and relatively low cost, of testing for indicator organisms encourages more frequent testing, which can allow bacterial contamination to be detected at a higher frequency. In order to be considered an "ideal" indicator by the WHO, an organism must:

1) Be universally present in feces of human and animals in large numbers.
This is needed to ensure that the organism is present in levels that are detectable by reasonable measures.
2) Not able to multiply in natural waters.
Should an indicator organism have the ability to not only survive, but to multiply in natural waters, there would be no way to determine if the organisms present in a sample were of natural, or human-gut origin.
3) Persist in water in a similar manner to fecal pathogens.
Indicator organisms should survive in environmental conditions similar to those in which pathogens survive and should not survive in other environmental settings.
4) Be present in equal or higher numbers than fecal pathogens.
Again, for detection purposes, indicator organism should be present in, at minimum, the same quantity as the pathogens present in a sample.
5) Respond to treatment in a similar fashion to fecal pathogens.
In order to use indicator organisms to test the efficacy of a treatment process, their response to treatment must mimic that of actual pathogens.
6) Be readily detectable by simple, inexpensive methods.
If testing for indicator organisms is not significantly less complicated, and expensive, than full pathogen testing, they become useless as a tool for determining microbial drinking water quality in developing countries.

3.2 Indicator Methods

When checking for the presence of an indicator organism in a sample, there are three methods scientists commonly employ. Each of these methods can be used to check for the presence of various indicators and are used in almost all commercially available water testing products.

3.2.1 Presence-Absence

A presence-absence (P/A) test is the simplest method of testing, as it is not a quantitative assessment of the contamination level. Instead, by adding a water sample to a selective growth media, a user is able, after 24-48 hours of incubation, to determine whether or not contamination is present in the sample. Should the result be positive, often a P/A test is followed by a more rigorous enumerative method.

The broth most frequently used consists of lauryl sulfate-trypose and lactose, with some industry producers attaching a fluorescent tag so the presence of both coliforms and *E. coli* can be determined in a single step (Micrology Laboratories, 2008).

3.2.2 Membrane Filtration

The membrane filtration method allows users to enumerate the number of coliforms in a sample by passing a given amount of the water sample through a small (0.45 micrometer) membrane filter. The filter is then placed in a dish containing growth medium and incubated for 24 hours. The exact incubation period and temperature will vary based on the type of bacterial contamination being tested. Coliforms and *E. coli* can both be cultured with this method depending on the presence of the appropriate sugar dyes in the broth.

3.2.3 Most Probable Number

The most probably number (MPN) method makes use of statistical trends to infer, based on a series of P/A tests, the exact level of contamination. A typical example requires 3-5 test tubes containing various dilutions of the sample water mixed with a broth to be incubated and then examined for the presence of gas, indicating positive coliform growth. Using an MPN table, the total number colony forming units (CFUs) per 100ml can be determined. In many cases, the testing stops at this point, however to be thorough, a confirmation test should also be completed. A confirmation test consists of culturing some of the positive presumptive tests with an Endo-agar and noting the subsequent growth of colonies.

3.3 Current Indicator Organisms

3.3.1 Total Coliforms

The coliform group, consisting of gram negative, non-spore forming, rod shaped bacteria, has traditionally been trusted as the most reliable indicator for drinking water in industrialized nations. Coliforms also are able to ferment lactose within 24-28 hours when incubated at 35°C, a feature that is helpful in identifying them among other bacteria. Coliforms are also the broadest category of organisms used as an indicator, meaning that a variety of species are used to identify the potential presence of contamination. Often the presence of total coliforms simply indicates that

further, more specific testing is required. The species included in the coliform group include, but are not limited to, *Escherichia, Citrobacter, Enterobacter, Klenseilla, Enterobacter cloacae and Citrobacter freundii* (Gerba, 2000).

3.3.2 Thermotolerant Coliforms

Thermotolerant coliforms are a subset of the total coliform group. The coliform species considered part of this subset are only those that have the ability to ferment lactose at a temperature of 44.5° C. Often the term "thermotolerant" is used interchangeably with "fecal," incorrectly combining temperature and origin classifications. Given that a number of natural environments exist that maintain temperatures as high as those found in the human gut, it is important to use the terminology correctly as to not confuse the implications of an indicator. *Escherichia, Klebsiella, Enterobacter* and *Citrobacter* species all fall into the sub-category of thermotolerant bacteria (Gerba, 2000).

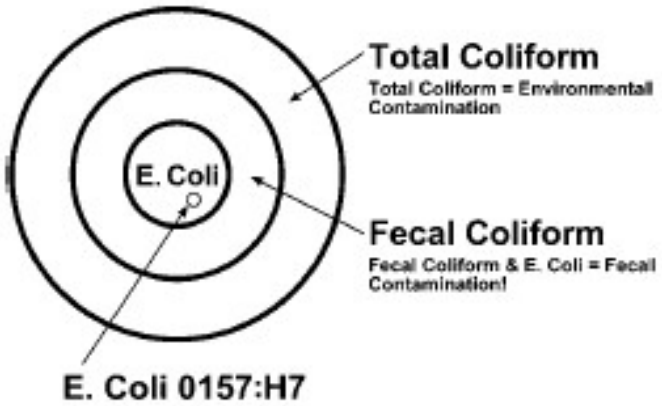
3.3.3 Fecal Coliforms

Fecal coliforms are a more defined subset within the thermotolerant coliforms group. Many of these organisms are physiologically similar to their parent set, however their origin is know to be the gut of a human or other warm-blooded animal species.

3.3.4 Escherichia coli

Escherichia Coli, commonly known as *E. coli,* is a single species subcategory of fecal coliforms. There are many strains of *E. coli*, only a small fraction of which cause disease. Most commonly it is strain O157:H7 that is to blame for severe cases of breaches in public health (Washington State Department of Health, 2011). However, the presence of any strain of *E. coli* is likely indicative of fecal contamination of the water source and further testing is required.

Figure 7. Total Coliforms, Fecal Coliforms and *E.coli*



(Washington State Department of Health, 2011)

3.4 An Evaluation of Current Indicators

To date, no organism has been identified that perfectly fulfills the criteria set out by the WHO for an "ideal indicator." Total coliforms and *E. coli* are the most commonly used indicator organisms, however both of these species have shortcomings that affect their accuracy for use in assessing drinking water quality.

Coliforms have been demonstrated in several studies to persist, or ferment lactose, independent of warm-blooded hosts, in environmentally pristine tropical conditions (Hazen, 1987). This observation makes coliforms non-ideal indicators, particularly in tropical environments, where water-borne diseases have a particularly high prevalence (Gray, 2003). In some cases, coliforms have also been found to be more robust than the disease causing bacteria themselves, continuing to reproduce despite the employment of treatment methods (Gray, 2003). Finally, current tests methods are unable to distinguish between bacteria originating from the human gut versus that of other mammal species (Chengwei Luo, 2011).

Recent studies from the Georgia Institute of Technology have drawn into question the appropriateness of *E. coli* as an indicator organism in a wide range of environmental conditions. Researchers have identified nine unique strains of *E. coli* that have adapted to survive independently in the environment (Chengwei Luo, 2011). A number of these strains exist is soil ecosystems which, when flooded with heavy rains, could easily mix with surface or ground water sources. This contamination, by naturally occurring *E. coli*, could lead to the mismanagement of safe water sources. In regions where water scarcity is an issue, an increase in false positive microbial tests could have a highly negative affect on the health of the community.

Despite the shortcomings discussed in the aforementioned indicators, the World Health Organization uses these organisms to set and assess drinking water quality.

3.5 Motivation for Further Study

In addition to the uncertainties surrounding the use of coliforms and *E. coli* as indicator organisms, there are a number of difficulties associated with the testing procedures themselves.

- **Complexity**

The municipal drinking water standards in the United States to date are widely monitored by the IDEXX Quanti-Tray® and the Millipore Membrane Filtration consumer products. Both methods measure the level of contamination by determining the number of total coliforms and *E. coli* present, yet both testing procedures involve multi-step processes, leading to an increased risk of contamination of the sample. To perform the Quanti-Tray® test, samples must first be mixed with the Colilert substrate in a 100ml sterile bottle and transferred into a tray which is then heat sealed before being incubated. The details of the Quanti-Tray® procedure can be found in Appendix F.

In membrane filtration, care must be taken to ensure that filter is sterile before use and that the pressure of sample water through the filter is adequate to move the sample but not too strong as to tear the filter. The method also requires re-sterilization of the apparatus between each test, making the process very time intensive, expensive and inappropriate for third world settings.

- **Resource Availability**

Areas of the world where microbial tests are most needed, namely developing countries, are often not equipped with modern laboratory equipment or trained technicians. Remote areas may not have consistent, or in many cases any access, to electricity that is needed to power the incubators, refrigerators and other devices to execute the most accurate testing methods. In the rare cases where equipment is accessible, malfunctions often cut short the life of the product as the repair skills required are not locally available.

- **Financial Burden**

Large amounts of capital are needed to establish and maintain laboratory facilities over a long period of time. A single Quanti-Tray® Sealer, for example, can cost $25000. There are also recurrent costs of water tests that are made for one time use and can range from $1-20 per test. As water testing does not actually improve water quality, many donors and local governments themselves tend not to invest heavily in these efforts.

It is clear from the discussion of standard indicators that exploration into new methods of assessing microbial water quality is warranted. As outlined in the study objectives, this thesis will evaluate H_2S and Easygel® as individual tests and as a combination to determine drinking water quality. The EC-Kit, containing the 10 ml pre-dispensed Colilert and the 1ml Petrifilm™ test, will also be evaluated for the same criteria. Particular attention is paid to the H_2S test as it relies on hydrogen sulfide producing bacteria as the indicator organism as opposed to the more traditional coliform based proxies.

4. Hydrogen Sulfide (H_2S) Producing Bacteria

4.1 The Metabolism of H_2S Producing Bacteria

Hydrogen sulfide (H_2S) producing bacteria are a distinct category of prokaryotes due to their ability to use sulfate as a terminal electron acceptor in respiration. The primary end products of this type of respiration are H_2S and CO2, found in a ratio of 1:2. Under special conditions, several other end products, including H2 and methane, are possible, however they are not consistent across H_2S producing bacterial metabolisms (Madigan, 2006). The process is considered a type of dissimilatory sulfate reduction and produces large amounts on energy for the cell.

The alternative test being evaluated in this study is concerned with identifying the presence of hydrogen sulfide producing bacteria in the environment. It does so by detecting H_2S, previously identified on of the metabolic products of this type of organism.

4.2 H_2S in the Global System

In order to evaluate H_2S producing bacteria as potential indicator organisms, it is necessary to understand the role and presence of sulfate in the global system.

H_2S producing bacteria are more prevalent in some environments than in others due to the fact that there are several compounds that are more energetically favorably electron acceptors. According to the Gibb's free energy of these compounds, or how favorable the reaction is to proceed in the forward direction, oxygen, iron, nitrate and manganese will all be utilized before sulfate will be consumed, and H_2S produced. Thus, in environments where these elements are present in substantial quantities, it is expected that fewer H_2S producing bacteria will be present.

Figure 8. Gibbs Free Energy of Terminal Electron Acceptors

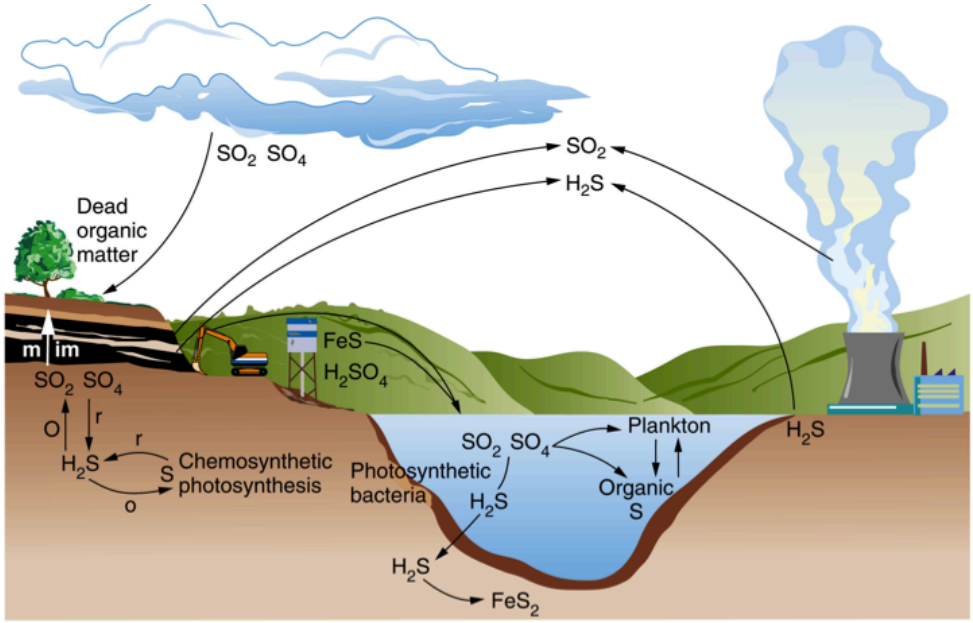
Reactions for Electron Acceptors	ΔG (kJ/eq)
Oxygen $¼\ O_{2\,(g)} + H^+ + e^- = ½\ H_2O$	-78.72
Ferric Iron $Fe^{+3} + e^- = Fe^{+2}$	-74.27
Nitrate $^1/_5\ NO_3^- + {}^6/_5\ H^+ + e^- = {}^1/_{10}\ N_{2\,(g)} + {}^3/_5\ H_2O$	-72.20
Manganese $½\ MnO_{2(s)} + 2H^+ + e^- = {}^1/_2\ Mn^{+2} + H_2O$	-38.89
Sulfate $^1/_8\ SO_4^{-3} + {}^{19}/_{16}\ H^+ + e^- = {}^1/_{16}\ H_2S_{(aq)} + {}^1/_{16}\ HS^{-+} + ½\ H_2O$	20.85

As modified from (Chisholm, 2010)

By this logic, it stands that H_2S producing bacteria are likely to be found in and around hydrothermal vents, marine sediments, saline microbial mats, oil fields and anaerobic wastewater treatment plants. Since H_2S has a low solubility product, tests for the chemical are likely to be sensitive even when concentrations of H_2S producing bacteria are low.

The circulation of the sulfate is governed by the sulfur cycle as is depicted in Figure 9. As shown, sulfur is one of the most ubiquitous elements in most natural environments; however, the major reservoirs of the cycle are in the atmospheric and lithospheric components of the cycle (Chisholm, 2010). The presence of H_2S producing bacteria in soil components has the potential to produce false positives during microbial testing and should therefore be kept in mind in interpreting the results of field-based H_2S tests.

Figure 9. The Sulfur Cycle



(Chisholm, 2010)

In looking at the processes that take place throughout the cycle, there are three in which H_2S is either produced or is present in an intermediate step. These are 1) mineralization of organic sulfur 2) oxidation of elemental sulfur and 3) reduction of sulfates in sulfide (Chisholm, 2010).

4.3 The H_2S Test

4.3.1 Origins of the H_2S Test

From 1946 to 1975, Allen and Geldreich analyzed reported outbreaks of water-borne diseases, and found that, in over 50 percent of cases, contaminated ground water was the cause for these lapses in public health. The pair observed that the insensitivity of current coliform detection methods was allowing water that contained harmful pathogens to pass through water supply systems unnoticed. Their 1975 paper, "Bacteriological criteria for ground water quality," articulated the need to develop improved bacterial detection methods (Geldreich., 1975).

These observations prompted Manja et al., during an outbreak of Hepatitis A in India, to develop the H_2S test to detect fecal contamination in drinking water supplies. Water samples were

simultaneously tested using the standard laboratory MPN method for coliforms and the new H_2S P/A paper strip method (Manja M. M., 1982).

In order to indicate this presence of H_2S in a sample, a prepared media containing bacteriological peptone, dipotassium hydrogen phosphate, ferric ammonium citrate, sodium thiosulphate and sodium lauryl sulfate was added to a water source via dried paper strips. If there was H_2S present, a reaction produced iron sulfide, an easily identifiable black precipitate. Accompanying the black color was a strong, potent smell described in many sources as that of a "rotten egg."

Samples with resulting coliform counts greater than 10 CFUs per 100ml in the standard MPN method for coliforms, were considered unsafe to drink, as were 20ml H_2S tests in which sample water turned black. Black, or positive results, from the new method were further cultured on nutrient agar to determine the taxa of H_2S producing organisms present.

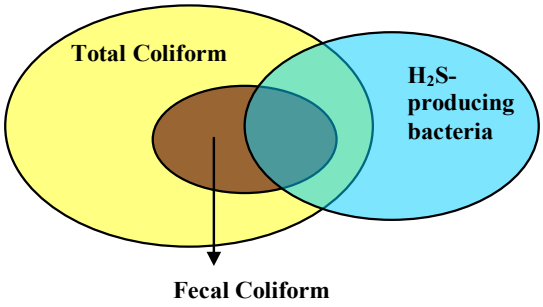
Of the 699 laboratory analyzed samples, all sources with over 40 CFUs per 100ml by MPN standards were also deemed unsafe by the H_2S test, demonstrating the new indicator organism as viable option in emergency or resource scarce situations (Manja M. M., 1982). Overall, the new test demonstrated agreement with the standard method in 88.34 percent of tests. The most common forms of bacteria identified via the culture method were *Citrobacterfreundi, Salmonella, Proteus* and H_2S producing strains of *E. coli.*

Since the publication of these results in 1982, investigations as to the applicability of the H_2S test for various environmental conditions and contaminant levels have been ongoing. Further experimentation with the test media has also explored ways to improve the accuracy and reliability of the method.

4.3.2 H_2S Test Correlation with Fecal Coliforms

Figure 10 below shows that while H_2S producing bacteria do overlap with both total and fecal coliforms, there are a number of species of producing H_2S that are not correlated with the traditional indicators of disease pathogens. Despite this fact, numerous studies detailed in this section establish a strong correlation between H_2S producing bacteria and fecal coliforms.

Figure 10. The relationship between H_2S producing bacteria and coliforms



(Low, 2002)

In 1989, as a part of a larger study aimed at identifying and verifying a single, simple microbiological test for drinking water quality, Ratto et al. undertook a study of five drinking water distribution sites in Lima, Peru (A. Ratto, 1989). The H_2S paper strip test was among three bacteriological tests, run on all samples to provide a basis for comparison against the "New Test," in this case coliphage, results (Clark, 1968).

Outcomes of the study concluded that the H_2S test was at least as accurate as the MPN method for both for total coliforms and *E. coli*. Ratto specifically recommended that the H_2S test be used in rural environments where access to full laboratory facilities was unavailable (A. Ratto, 1989).

Kromoredjo & Fujioka also set out to determine an appropriate microbial test for drinking water quality and focused their work on a water distribution system in Banjarmasin, Indonesia. The pair evaluated three test methods; the 20ml H_2S paper strip test, the lauryl tryptose and 4 methyl-umbelliferyl-B-d-glucoronide (LTB+MUG) test, and the MPN method for total coliforms and *E. coli* (P. Kromoredjo, 1991). In this case, all of the bacterial tests performed similarly, again indicating a consistency between the H_2S test and the standard MPN method. The authors highlighted the usefulness of the H_2S test for ease of use features including cost, lack of electricity required, and short incubation periods.

In these, and other, studies the efficacy of the H_2S test is not effected by the presence of, or interaction with, non-H_2S producing bacteria as was demonstrated by Grant and Ziel (Low, 2002) (Grant Z. , 1996).

Overall, research conducted over the past 25 years, across several continents, has indicated a high degree of correlation between H_2S producing bacteria and fecal coliforms (P. Desmarchelier, 1992).

4.3.3 Taxa identified by the H_2S Test

Manja, in his original study, identified and cultured the H_2S producing bacteria. The bacteria identified, in order of prevalence, were *Citrobacter freundi* (62 percent), *Salmonella species* (16

percent), *Proteus mirabilis* (5.5 percent), *Arizona* species (5.5 percent), *Klebsiella species* (3 percent) and various H_2S producing strains of *E. coli.* The culture method for taxa identification is fundamentally flawed as only a small fraction of bacterial species can be grown in culture and thus, the list bacterial species produced by Manja et al. is likely incomplete. Further genomics based identification is necessary if all present species are to be identified.

4.3.4 H_2S Applicability Across Water Sources

The focus of Nair's 2001 study was on the applicability of the H_2S test to check various sources of drinking water. Rainwater, borehole water, catchment water and aboriginal community water samples were all tested with the H_2S test, as well as standard procedures. Results indicated high levels of accuracy in both treated and untreated water samples. In developing countries where levels of accepted total coliforms are often slightly higher, on the order of 10MPN per 100ml, H_2S was concluded to be a valid method (J. Nair, 2001).

4.3.5 Temperature and the H_2S Test

Castilla et. al. conducted a study of "Disinfected and Untreated Drinking Water Supplies in Chile by the H_2S Strip Test" (G. Castillo, 1994). The purpose of this exploration was to evaluate the applicability of the test specifically for tropical and sub-tropical environments, as temperature is significant factor in the usefulness of coliforms as indicators. In treated and untreated drinking water, the 100ml H_2S tests consistently indicated 10 percent more contaminated samples than the Quanti-Tray® method. All positive H_2S tests were cultured and 81 percent were found to contain coliforms at an incubation temperature of 32°C; at 35°C, 85 percent were found to contain coliforms. 85 percent of sample cultures were also found to contain known pathogens including strains of *Clostridium* (G. Castillo, 1994). The variation in the number of positive, or contaminated, reported samples was accounted for by heterogeneous distribution of indicator organisms in the water sources. Castilla et al. concluded that the H_2S test could be applied to tropical regions (G. Castillo, 1994).

Work by Pillai et al. at Murdoch University, corroborated these findings, concluding from their research that the H_2S test was generally effective between 22-44°C (J. Pillai, 1999).

4.3.6 H_2S Test Modifications

In 1996, Grant & Ziel faced a resource challenge that led them to also experiment with the composition of the H_2S test media. At this time, one of the original ingredients used by Manja et al, Teepol, was no longer commercially produced. Instead, the researchers replaced the ingredient with a chemical with similar properties, lauryl sulfate sodium salts, and tested water in 100ml sample sizes (M. Grant, 1996). An additional difference in the procedure came in the form of the substrate. Instead of absorbing the H_2S media onto paper strips, a six times concentrated liquid medium was used. Despite these differences, Grant and Ziel found that H_2S was a valid alternative method for the assessment of microbial drinking water quality.

Venkobachar et al. experimented with a slightly altered version of the H_2S paper strip test that included L-cystine, in addition to the original media, in 20ml volumes (C. Venkobacar, 1994). Testing in India revealed that paper strips, including the additional ingredient, were more accurate in determining the bacterial water quality of a sample. In addition the modified media reduced the incubation time needed before results could be interpreted.

Pillai et al. made similar modifications to the H_2S test media to determine if incubation time could be decreased by adding yeast extract or varying the ingredient concentrations (J. Pillai, 1999). L-cystine was added to the media and again an increased sensitivity was observed. This substrate composition is now knows as the M2 media (M2) and was used in this study.

4.3.7 H_2S Test Combinations

In 2010 Chuang, Trottier and Murcott published a "Comparison of four field-based microbiological tests" that examined laboratory made H_2S paper test strips with a range of sample volumes, the Easygel® test, the Colilert test and the Petrifilm™ test. Test performance was analyzed using drinking water samples collected in the Philippines and Cambridge, Ma in the winter and spring of 2010. The results indicated that none of the tests individually was suitable for assessing water quality, but that the combination of the Colilert and Petrifilm™ yielded less than a 6% error as compared with standard methods The combination of the 20ml H_2S test and Easygel®, another enumerative test for coliforms and *E. coli,* showed the promise with a 0% error however a small sample size limited the confidence level in these results (Trottier, 2010). It is Trottier's result that is the basis for the current investigation into new microbial test combinations.

5. Research Methodology

5.1 Overall Research Plan

The overall research plan was completed from December 2010 through December 2011. The major steps, are described in detail in the following sections and include: A literature review of the development and performance of the H_2S test; preliminary laboratory training and media preparation; development of overall sampling plan and household interview questions; field testing of five microbial tests and collection of household interview data; data review and analysis.

5.2 H_2S Literature Review

A literature review of the H_2S test was completed in the fall of 2011 in order to determine the most germane work that could be completed during the sampling period in Ghana. The review spanned the development of presence/absence methods by Clark (1968) to a recent study by Chuang, Trottier and Murcott in the Philippines (2010). For comparison purposes, the methodology and composition of testing substrates in this study, closely follows that of Chuang et al.

5.3 Laboratory Training and Preparation

In November of 2010, the author completed laboratory trainings in Lecturer Susan Murcott's environmental engineering lab at MIT. Membrane filtration, Quanti-Tray® and EC-kit techniques were learned and practiced to ensure their correct execution in the field.

In December 2010, following the procedure of Venobachar (1994), the H_2S M2 media (M1+ L-cystine) was prepared in strip form. M2 media was selected due to its demonstrated ability to increase sensitivity and accuracy in testing of rural water sources (C. Venkobacar, 1994) (J. Pillai, 1999).

To check that the laboratory prepared H_2S test strips did in fact correlate with standard methods of testing they were verified against Quanti-Tray® results for Charles River Water, known to be generally contaminated, and Cambridge tap water, a "pure" source. The results of these initial H_2S tests did in fact correlate with those attained using standard methods, however, they are not included in the data set of this study.

Forty-two prepared M2 H_2S dry strips were packaged and transported to Ghana in sterilized glass bottles. Over 100 additional dry strips were also prepared for future use. Finally, a dry ingredient mixture of the M2 substrate was brought to the field laboratory in a plastic bottle should additional strips need to be made on location.

5.4 Field Work and Collection Methods

During the month of January 2011, the author sampled 111 unique water sites from a both of improved and unimproved sources in and around Tamale, Northern Ghana. Microbial tests were performed at the laboratory in the Pure Home Water house/office at Kalpohin Estates in Tamale. Laboratory equipment, including an incubator and Quanti-Tray® sealer, was borrowed from the Innovations for Poverty Action (IPA) lab in Tamale in order to complete all necessary microbial tests. Although the author originally intended to complete 50 testing sets, where a set refers to the completion of the 20ml H_2S test, the 5 ml Easygel® , the EC-Kit, the *QuantiTray*®, and turbidity and pH tests, limited supplies caused variation in the number of tests that could be executed with each of the microbial methods. More detail regarding each of these microbial methods can be found in the next section.

Figure 11 pinpoints the various locations around Tamale where water samples were obtained. The sites include both villages where both water samples were collected and household interviews completed (11), villages where only water samples were collected (2), as well as a polluted lake from which multiple communities draw water (1).

Figure 11. Locations of Sample Collection

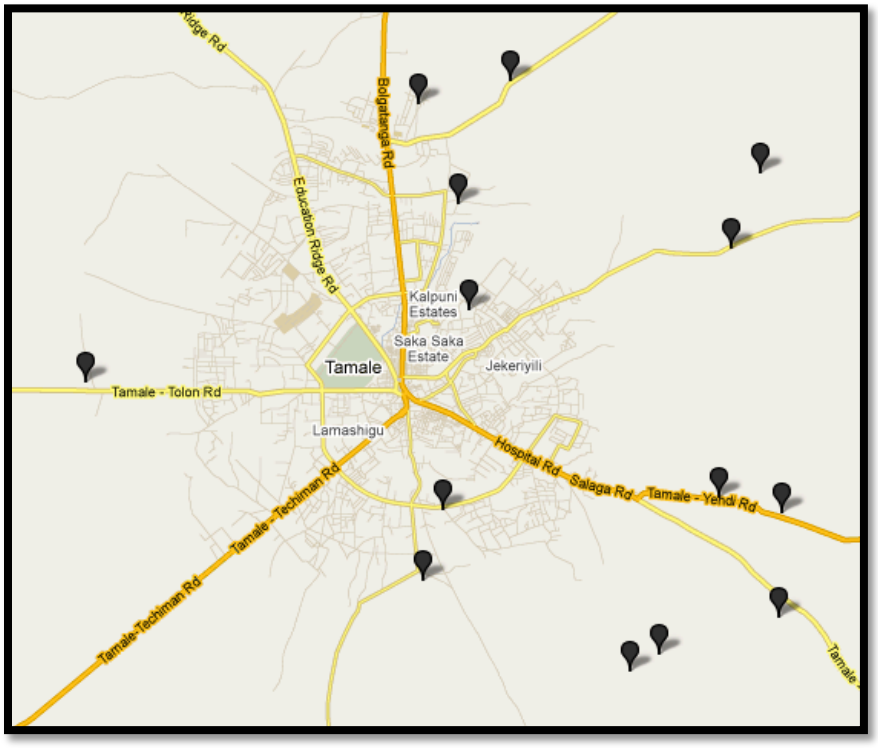


Figure 12. Collection Sites for Microbial Testing

Date	Village	Samples Collected
1/4/11	Taha	8
1/5/11	Kalpohin	8
1/6/11	Gbalahi	8
1/7/11	Kpanvo	8
1/8/11	Kasaligu	8
1/10/11	Banvim	8
1/10/11	Kplebilla	1
1/11/11	Wuvogo	8
1/12/11	Tunayilla	8
1/13/11	Wamale	8
1/14/11	Chansie	7
1/17/11	Parishe	7
1/18/11	Kotingle	8
1/19/11	Gbanyamn	8
1/20/11	Wayamba	8

5.5 Microbial Test Methods

5.5.1 Colilert

Colilert, produced and sold by IDEXX, makes use of the enzyme substrate method, which is approved by the U.S. EPA and is listed in the Standard Methods for Examination of Water and Wastewater. The Colilert detection limit is set at 10 MPN/100ml for the 10ml pre-dispensed sample size (IDEXX, 2011).

The enzyme substrate standard method takes a different approach from traditional media where both targeted and non-targeted organisms are grown on a nutrient-rich growth media. Instead, the enzyme substrate method utilizes nutrient indicators, ONPG and MUG, which act as the primary sources of carbon in the metabolisms of coliforms and *E.Coli*. The metabolism of ONPG by the enzyme β-galactosidase in coliforms causes the color of the bacterial cells to change to yellow. Similarly, as *E.Coli* metabolizes MUG with β-glucuronidase, it fluoresces. These visual cues, yellow color and fluorescence, allow us to infer the presence of coliforms, *E. coli,* or both in a water sample. This methodology is part of the patented approach called "Defined Substrate Technology" (IDEXX, 2011).

In this study, Colilert was used in a presence/absence format testing 10ml samples of drinking water. Full procedural details for the Colilert test can be found in Appendix E. As it was available at the time of field collection, an electric incubator was used as opposed to a waste-belt incubator, which is described in the procedure.

5.5.2 Petrifilm™

Petrifilm™ *E. coli*/Coliform Count Plates are used to quantitatively assess the presence of total coliforms and *E. coli* present in 1ml of the collected drinking water samples. Petrifilm™ is comprised of a nutrient-rich media that provides a food source for bacteria to grow. Also in the media are specific indicator sugars that when metabolized produce either a red, for total coliforms, or blue, for *E. coli*, color. A covering film also traps gas (CO2) produced by each of the metabolic processes, further simplifying the process of reading results (3M, 2011).

Used together with the Colilert test, the pair is known as the EC-Kit and was developed by Professor Robert Metcalf of California State University at Sacramento, in association with Susan Murcott of MIT. The EC Kit has been tested and verified against standard methods (Chuang T. M., 2011). Directions for execution of the Petrifilm™ test can be found in Appendix E.

5.5.3 Easygel®

Easygel®, manufactured by Micrology Labs, utilizes an agar replacement called pectin-gel which serves as a nutrient source for cultured bacteria. The product comes in two parts: a liquid medium that must be stored frozen before use, and a pretreated petri dish. Water samples are mixed with the media after it has thawed for a period of 12 hours. When the media and water mixture are added to the dish, ions diffuse into the pretreated chemical layer and form a recognizable gel. This method eliminates much of the hassle associated with making and transporting agar plates (Micrology Laboratories, 2011).

The Easygel® has linked sugars enabling users to enumerate the number of total coliforms and *E. coli* in the sample. Once a gel layer has formed on the plate, samples are incubated at room temperature or a higher controlled temperature. After 24 hours, samples can be read for total coliforms, which will appear as pink dots, and *E. coli*, which will appear as blue or purple dots. Full details on the execution of the Easygel® test as well as interpretation of results can be found in Appendix D.

5.5.4 The H_2S Test

The 20ml presence/absence H_2S test, as described in significant detail in Chapter 4, was conducted using laboratory made paper test strips. The H_2S test can be incubated at ambient temperature, is highly portable and produces a black smelly result making it intuitive to associate with poor water quality.

Figure 13 below is a side-by-side comparison of a "blank" sample where H_2S is not present (left) and a "positive" sample where H_2S is present, indicating potential fecal contamination. Clear visual cues are yet another example of the potential benefits of using this new test.

Figure 13. H_2S Results

5.5.5 Quanti-Tray®

Quanti-Tray® is an EPA-approved, highly reliable and accurate method of testing drinking water quality. In this study it is used as the standard against which all "New Tests" are measured.

Quanti-Tray® uses the Colilert substrate described in Section 5.5.1, however instead of using a pre-dispensed 10ml glass bottle, a tray with 97 wells enables the enumeration of the level of contamination present. Using 100ml of sample water, the 97 wells are filled and the tray then sealed and incubated for 24 hours. Using the statistical method of Most Probably Number (MPN), the number of the coliforms and *E. coli* in the original sample can be inferred. Due to the high number of wells, Quanti-Tray® is able to produce results with 95% confidence limits. The sensitivity of the test ranges on the low end from 1MPN/100ml up to the very high contamination level of 2149MPN/100ml (IDEXX, 2011).

IDEXX produces several different products all under the title Quanti-Tray®. These products vary only in the number of wells present in the tray, determining the range of the MPN that can be determined using the method. The specific product used in this experiment is the Quanti-Tray®/2000.

5.6 Field Interviews

Microbial water testing is critical to ascertain the precise of degree of contamination in a water sample, however results can be misleading. A sample represents only the contamination present in a small fraction of the source at a single point in time. In 2010, Patrick et al, emphasized the importance of performing sanitary surveys in conjunction with water testing to alleviate these issues of scale. Household sanitary surveys can inexpensively provide context and a broader understanding of the local factors contributing to the drinking water quality (J. M. Patrick, 2011).
The motivation for collecting interview data was three fold; first, in order to ascertain the primary location of drinking water collection in village and the reliability of that source; second, to identify what household storage and treatment methods, if any, were common and perceived to be effective; and third, to become familiar with common health problems or trends related to water quality existed.

In this study, household interviews were conducted as opposed to full sanitary surveys due to time and resource constraints. Interview results are meant to provide context to microbial test results in the greater Tamale region and should not be extrapolated to Ghana as a whole.

5.6.1 Interview Topics

An overview of the themes covered is presented below with a full list of interview questions available in Appendix H.

Water Source/Storage

Given that the first source of contamination for drinking water can be the point of extraction, it was critical to gather information surrounding where families gather water. Sources range from dugouts, to community wells, to purchasing water from their neighbors with piped sources. Questions were asked surrounding collection and storage containers that could also serve as points of contamination. Questions were also asked regarding the maintenance and cleaning of the storage container.

Water Treatment

With a baseline of starting water quality established, respondents were asked if they currently, or have ever, employed any treatment methods to their water before consumption. Examples were provided including a guinea worm filter, alum and chlorine. How they came to use those methods was an additional topic; if they were given the tools by an outside organization, if they sought out treatment themselves, etc. If possible, information was also collected surrounding their knowledge of the effects of various treatment methods on the water quality.

Health/Sanitation

The final topic of the January interviews was health and sanitation practices. Use, and impressions of latrine use, was noted along with the occurrence of childhood diseases. An interview with Doctor Kwame of the health clinic near Wamale was also conducted. Official records of disease occurrence were attempted to be located, however local clinics and the national health ministry had only incomplete records. As a result, any data collected from these bodies is not referenced in this thesis.

5.6.2 Interview Data Collection

Responses were collected between January 3rd and 21st, 2011. Surveys were conducted informally, typically with the female(s) of the household. On average, 6 households were interviewed in 11 of the 14 villages visited over the month of January. At public sources where there was no individual present to interview, none was conducted, but general notes made on the appearance of the source quality and observed collection practices.

GPS locations and photographs were taken to complete the documentation.[4] The average interview time was approximately 15-20 minutes per household with responses manually recorded and later transcribed into a Google form.

Village Selection

Villages were chosen based on several contributing factors. First, based on location, villages were selected that were at a distance outside of central Tamale to be considered rural, but still close enough to be within a commuting distance. This also provided a good mixture of villages with and without access to piped municipal water.

The second factor to consider was the relationship of Pure Home Water, the host organization, with the community. Surveys tended to be most welcome in areas that were familiar with the work of the non-profit, and thus were preferentially chosen over those communities that were not.

In a similar vein, Ameen Hussein, the interview translator, had previously worked as Pure Home Water salesmen and his relationships proved invaluable in establishing rapport with local Chiefs as interviews were completed only after approval of the Chief was granted. In some cases, a request was made to limit the types of questions asked, or households approached, and this variability in questioning is reflected in the full interview results.

Household Selection

Households within a village were selected using convenience sampling, meaning that homes where women were currently present or that were within a range of a water source were preferentially interviewed as opposed to a true random sampling. This methodology is known to provide an approximation of the habits of the population and is commonly used in exploratory research (StatPac Inc., 2011). The results of convenience sampling can be extrapolated only to a narrowly defined population. In this situation, where the primary goal was to provide behavioral context to microbial results from specific households, this method of collection was appropriate.

Sources of Error

A potential source of error in the interview results has to do with information literally lost in translation. An individual not formally trained in field interview methodology translated and conducted interview. In several instances, it became clear, that the true meaning of the question was not conveyed to the interviewee. Efforts were made to clarify these misunderstandings, however responses in some cases were omitted from the analysis due to irrelevance.

[4] For more information on source documentation, please contact the author.

Table 3. An Overview of Household Surveys

Date	Village	Households Surveyed
1/7/11	Kpanvo	7
1/8/11	Kasaligu	5
1/10/11	Banvim	7
1/11/11	Wuvogo	6
1/12/11	Tunayilla	5
1/13/11	Wamale	6
1/15/11	Chansie	6
1/17/11	Parishe	6
1/18/11	Kotingle	5
1/19/11	Gbanyamn	7
1/20/11	Wayamba	7

6. Data Analysis Methodology

The main goal in performing a statistical analysis is to verify the accuracy of H_2S and Easygel® tests as individual presence/absence and enumerative indicators for microbial contamination, as well as to determine statistical improvement in accuracy when the two are used in combination as compared to a standard method, IDEXX Quanti-Tray®. In addition, the 10ml pre-dispensed Colilert and Petriflm are also evaluated individually and as a pair for their efficacy in detecting *E. coli* in collected water samples. Finally, where appropriate, results of the analysis are compared with those obtained by previous studies to determine if performance is consistent across the varying environmental conditions**.**

Results were analyzed using Microsoft Excel. The statistical methods employed are consistent with those performed in Trottier (2010), as well as the 2011 paper by Chuang, Trottier and Murcott "Comparison and Verification of Four Field-Based Microbiological Tests Against Quanti-Tray®." The following sections detail the specific statistical tools and methods employed in the analysis.

6.1 Statistical Tools

6.1.1 Contingency Tables

A contingency table, or as cross tabulation table, is a statistical tool often used when comparing results that can be classified into two distinct categories, A and B. Each category has within it multiple unique results i.e. A1, A2, etc. Table 4 demonstrates the matrix of potential outcomes resulting from categories A and B.

Table 4. Example Contingency Table

	B1	**B2**
A1	a	b
A2	c	d

In this analysis, "A" will typically represent one of the "New Tests" and B, the standard method. Manipulation of this matrix will be used to determine statistical independence between two identified criteria A and B. Cells containing values a, b, etc., indicate the number of observed occurrences that meet the criteria of the two categories and is referred to as a result cell. Table 5 below depicts an example contingency table for the 20ml H_2S test and Quanti-Tray®.

Table 5. Quanti-Tray® vs. H_2S Contingency Table

	Quanti-Tray®	
H_2S Test	**Presence**	**Absence**
Presence	85	0
Absence	11	15

From this depiction it is simple to determine if the two variables, A and B, have contingency, which is to say that the number of results in a column vary significantly over the rows. If results in a column are relatively consistent over the rows then it is likely that the variables, A and B, are do not have contingency, that is, any differences could be attributed to chance (Kirkman, 2011) (Lowry, 2008). A full list of the contingency tables created in this study can be found in Appendix A.

6.1.2 Chi Squared Test

Using the result cells in a contingency table, the chi-squared test can be used to quantitatively determine if the relationship between variables can be attributed to chance. The test operates with the base assumption of the null hypothesis, meaning that it is initially assumed that there is no relationship between the variables and any pattern in the result can be attributed to random chance. Following this logic, the greater the chi squared value, the greater the statistical correlation between the two variables.

Equation 1. Chi-Squared Equation

$$X^2 = (Observed - Expected)^2 / Expected$$

It is important to recognize that the chi-squared test cannot be used to calculate statistical relationships between variables if the expected frequency is less than five as determined by a 2x2 contingency table. In a chi-squared test, it is necessary to assume that the data follows a Gaussian distribution in order to simplify the statistical calculations (Lowry, 2008). This distribution assumption falls apart with extremely low expected values.

6.1.3 Fisher's Exact Test

A Fisher's Exact Test is an alternative statistical method for determining the significance of the contingency of experimental variables. Unlike a chi-squared test, a Fisher's exact test makes no assumptions with regard to the distribution of data. It is therefore highly appropriate for data sets where contingency tables produce low expected result values. For this reason, the Fisher's exact test was the highly preferred method of analysis in this study.

The Fisher's exact test is calculated with the values derived from a 2x2 contingency table and follows the equation below (Lowry, 2008).

Equation 2. Fisher's Exact Test Equation

$$\Pr(a,b,c,d) = \frac{(a+b)!\,(c+d)!\,(a+c)!\,(b+d)!}{n!\,a!\,b!\,c!\,d!}$$

6.1.4 Statistical Significance

Statistical significance is a method of verifying the reliability of a produced result, or in other words, verifying that the result is unlikely to have been produced by chance. More specifically, establishing that a result is statistically significant requires calculation of the probability value (p-value), or degree of confidence. In chi-squared and Fisher's exact test, the null hypothesis that is assumed at the start of the analysis is that there is no relationship between the variables (Vassar College). Thus, with a calculated p value less than 0.05, the null hypothesis is rejected and is a relationship assumed. The significance of the presumed relationship varies with the p-value obtained. Specific guidelines for interpreting a range of p-values are included in the table below.

Table 5. Interpreting P-Values

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

6.1.5 General Statistical Methods

In keeping with the analysis performed by Trottier and Chuang, a number of general statistical tests were performed in order to provide further detail as to the accuracy and general performance of the microbial test results. The methods, abbreviations, definitions and equations with reference to the expected results in a 2x2 contingency table are given in Table 6 below.

Table 6. General Statistical Methods

Method	Abbreviation	Definition	Equation
True Result	TR	The percentage of samples for which the New Test produced the same result as the Standard Method.	(a+d)/(a+b+c+d)
False Positive	FP	The percentage of positive samples of the New Test that produced a negative result by the Standard Method.	b/(a+b+c+d)
False Negative	FN	The percentage of negative samples of the New Test that produced a positive result by the Standard Method.	c/(a+b+c+d)
Sensitivity	Sn	The capacity of the New Test to determine a true positive result as defined by the Standard Method.	a/(a+c)
Specificity	Sp	The capacity of the New Test to determine a true negative result as defined by the Standard Method.	d/(b+d)
Positive Predictive Value	PPV	The capacity of a positive New Test to predict the presence of *E.coli.*	a/(a+b)
Negative Predicative Value	NPV	The capacity of a negative New Test to predict the absence of *E.coli.*	d/(c+d)
Error	-	The sum total of all false results by the New Test.	(b+c)/(a+b+c+d)

6.2 Error Calculations

In order to calculate the error associated the results of the experimental microbial tests, Petrifilm™, Colilert, H_2S, Easygel®, as compared with Quanti-Tray®, a standard error calculation was completed. As is shown in Table 6, summing the false positive and false negative results, and dividing by the total number of tests, determined error.

In the case of water quality testing it is critical to not only calculate error, but also to think of it as it relates more broadly to the health impacts of an incorrect result. Referring to the result cells identified by "a, b, c, d" in a 2x2 contingency matrix, outcomes b and d are "false" in that in these cases the "New Test" did not produce the correct result as defined by the standard method. Although b and d tests are both incorrect, in practice they represent two very different situations, one a false positive and one a false negative. Questions related to which of these poses more of a threat to public health will be considered in the later chapters.

In addition, the Proportional Reduction in Error (λ), or PRE, is a tool used for determining statistically how much more accurate the prediction of an outcome becomes given an additional piece of information. In this case, the quality of the source water is the baseline assumption with the "New Tests" providing the additional information. Therefore the PRE will describe the improvement in predictive ability of the source water quality, given the outcome of a microbial test.

As was discussed in previous chapters, the UN distinguishes safe and unsafe drinking water using improved and unimproved source categorizations. Thus, the PRE of each of the various microbial tests is presented in terms their source water category, improved or unimproved.

To calculate the PRE of the microbial tests, a corresponding assumption table is created for each of the contingency tables. In the case of improved sources, the baseline assumption is that the source is safe and contains no microbial contamination. On the other hand, in the case of unimproved sources, the baseline assumption is that the unsafe and contains microbial contamination. Example assumption tables for the H_2S test are given below.

Table 7. Assumption Tables, H_2S

Assumption Table (H_2S) Improved Source

		Standard	
		Presence	Absence
Prediction	Presence	0	0
	Absence	22	4

Assumption Table (H_2S) Unimproved

		Standard	
		Presence	Absence
Prediction	Presence	74	11
	Absence	0	0

These assumptions are entered as "Error 2" in their respective PRE calculations. "Error 1" represents the error calculated using the experimental results frequencies. The more positive the value of the PRE, the greater impact the experiment test has on the ability to accurately predict contamination. The equation for calculating the PRE is given below.

Equation 3. Proportional Reduction in Error (PRE)

$$Proportional\ Reduction\ in\ Error = \frac{Error\ 1 - Error\ 2}{Total\ Error}$$

This methodology of error analysis falls under the category of Bayesian statistics that, unlike more traditional frequentist methods of analysis, assumes and incorporates inherently known information, called the "prior distribution," about the data set to begin with (Levine, 2011). In the case of this study, prior assumptions by the public as to the quality of improved or unimproved sources are paramount to determining in what circumstances the "New Tests" are appropriate.

6.3 Combined Test Analysis

In order to evaluate the effectiveness of two combined microbial tests with standard methods, results were correlated with WHO Risk Levels for drinking water quality. The levels for each of the microbial tests are listed in Table 8 below.

Table 8. WHO Risk Levels and New Test Results

WHO Risk Level	H_2S	Easygel® (CFU, Modified for a 5ml Sample)*	Colilert	Petrifilm™ (CFU)*	Quanti-Tray® (MPN)**
Conformity	-	0	-	0	<10
Low	-	0	-	0	<10
Intermediate	+	1-4	+	0	10-100
High	+	5-50	+	1-10	<100
Very High	+	>50	+	>10	<1000

*CFU indicates colony-forming units
**MPN indicates most probable number

Most probable number (MNP) is a statistical method for determining a likely number of colony forming units (CFUs) present. Though they are generated differently, with colony-forming units being physically counted in a sample, MPN and CFU values are both accepted methods to indicate contamination levels in drinking water samples.

From the WHO risk levels, a 3x3 contingency matrices can be constructed for both improved and unimproved water sources. Table 9 presents one example of such matrices for H_2S, Easygel® combination test. A full listing of 3x3 contingency tables can by found in Appendix A.

Table 9. Example 3x3 Contingency Table

H_2S +Easygel® Improved		**Quanti-Tray®**		
		Conformity/Low	Intermediate	High/Very High
H_2S +Easygel®	Conformity/Low	0	1	1
	Intermediate	0	0	5
	High/Very High	0	0	5

Additionally, assumption matrices are required to be in the same dimensions as the contingency table and thus a unique set of matrices were produced for this purpose. An example 3x3 assumption set, improved and unimproved is reproduced in Table 10.

Table 10. Assumption Tables, H2S +Easygel®

H_2S +Easygel® Improved		**Standard**		
		Conformity/Low	Intermediate	High/Very High
Prediction	Conformity/Low	0	1	11
	Intermediate	0	0	0
	High/Very High	0	0	0

H_2S +Easy Unimproved		**Standard**		
		Conformity/Low	Intermediate	High/Very High
Prediction	Conformity/Low	0	0	0
	Intermediate	0	0	0
	High/Very High	8	9	20

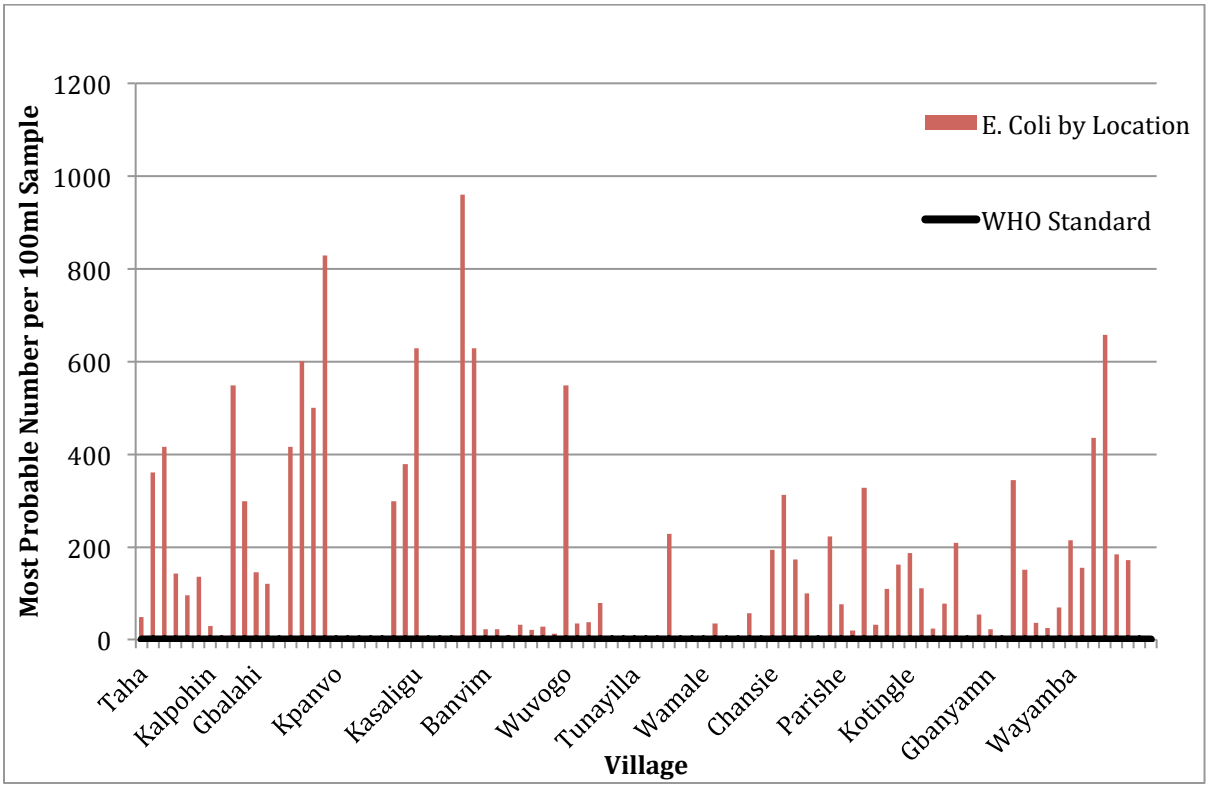
The categorization of results into WHO Risk Levels and the creation of assumption matrices simplifies a more complex data set into a format that is able to be manipulated using the same tools as was done with the 2x2 contingency tables.

7. Results

7.1 Overall Microbial Contamination

Overall, drinking water samples collected in and around Tamale, Northern Ghana were shown to be highly contaminated as indicated by the standard testing method, Quanti-Tray®. Only one village, Tunayilla, attained WHO conformity for *E. coli* presence with 0 CFU/100ml (World Health Organization, 2006). Figure 14, below shows the degree of microbial contamination as quantified by Quanti-Tray® tests performed at the Pure Home Water Laboratory in January 2011. As part of the discussion of contamination results, behaviors observed as part of the informal household interview process will be used to provide context to what may or may not be responsible for the measured quality outcomes. Given that *E. coli* are considered the most reliable indicator of fecal contamination, the analysis is this study will focus comparison of the New Tests with *E. coli* rather than total coliforms.

Figure 14. *E. coli* Contamination in Drinking Water Samples, Northern Ghana



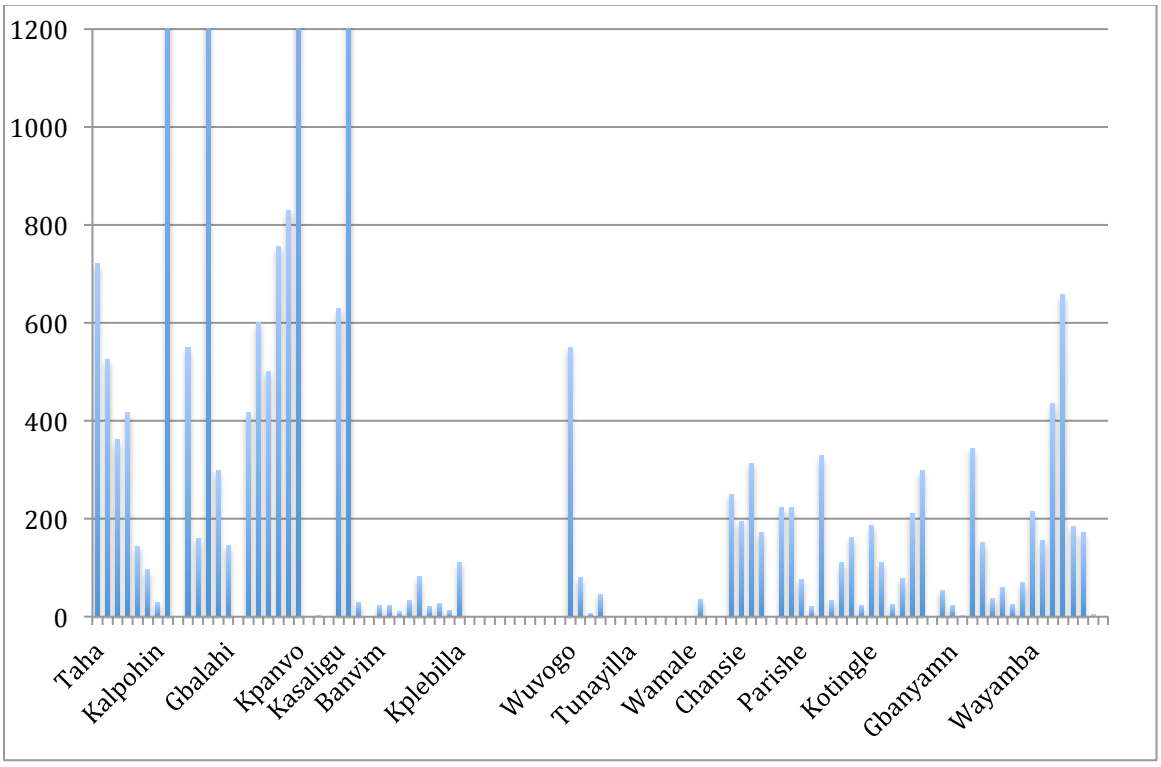
From Figure 14, it is clear that there is a range of contamination levels across villages, however almost all villages exceed the WHO standard for drinking water quality of 0 CFUs/100ml. Several villages, Kalpohin, Kpanvo, Tunayilla and Wamale, do display lower overall levels of *E. coli*. In all of these villages, at least some households had access to a supply of municipal piped water, which is expected to be of higher quality. The other villages in the survey relied solely on surface water sources, including dugouts and unprotected wells.

At a glance, there appears to be some correlation, in this study of Northern Ghana, between improved drinking water sources and safer drinking water. While an improved source does indicator better quality, data analysis reveals that 77 percent of samples from improved sources were found to be contaminated.

In the pilot study by the Joint Monitoring Program (JMP) for Water Supply and Sanitation, discussed in Chapter 1, 40 percent of improved sources had significant levels of microbial contamination. JMPs results are not unlike those of this study as although 77 percent of samples were contaminated, equating to approximately 45 percent of improved sources.

Moving to an exploration of unimproved sources in Northern Ghana, data indicates that just over 85 percent of samples contained *E. coli* at levels above the WHO recommended levels for safe drinking water. These contamination levels were also substantially higher than those found in improved sources as shown in Table 11.

Table 11. *E. coli* contamination in unimproved drinking water samples, Northern Ghana



7.2 H_2S P/A Results

Laboratory-made M2 H_2S media, in dry strip form, was used to test 111 unique water samples from the greater Tamale region. Samples were collected from a variety of sources including protected and unprotected wells, dugouts, traditional household vessels, safe storage containers, open dug wells, household and community taps. For the purpose of this analysis, results are either presented with regard to all H_2S samples, or by improved and unimproved sources as defined by Table 1. The contingency table for H_2S, as the "New Test," and Quanti-Tray®, as the standard method, is presented below.

Table 12. Contingency Table, 20ml H_2S vs. QuantiTray®

		Quanti-Tray®	
		Presence	Absence
20 ml H_2S Test	Presence	85	0
	Absence	11	15

The contingency table, Table 12, comparing H_2S and -®, indicates that, due to the result element "0," the chi-squared test is not appropriate. Instead, the Fisher's Exact Test was employed to obtain the statistical relationship between the H_2S test and Quanti-Tray®. This value, as well as the results of the general statistical methods can be found in Table 13.

Table 13. Statistical Results, 20ml H_2S vs. Quanti-Tray®

True Value	90%	Sensitivity	88%	Positive Predictive Value	100%
False Positive	0%	Specificity	100%	Negative Predictive Value	57%
False Negative	10%	Error	10%		

20ml H_2S vs. *Quanti-Tray*®
Fisher's Exact Probability: 0.0001
Extremely Statistically Significant

The results demonstrate a slight propensity, 10 percent, for false negatives, indicating that there was contamination present in a minority of samples that the M2 paper strip test did not detect. As compared to Trottier's work in 2010, the 20ml H_2S tests performed in Ghana were 6% more accurate by way of true results. Fisher's Exact Probability and the high True Value percentage give confidence to the H_2S as an indicator for the presence of *E.coli.* Sample sizes and calculated p-value indicate a high degree of confidence is appropriate for these results.

Previous literature, Chuang (2010) and Trottier (2010), indicated that the H_2S test is more likely to produce false positives due to the ubiquitous presence of hydrogen sulfide producing bacteria in natural environments. The conflicting results of this study in Northern Ghana attributed to the types of water sources sampled with few, less than 10, coming from underground sources. Another potential reason for this anomaly between the results is the environmental differences in the collection sites of water samples. Chuang and Trottier collected from rural sources in the tropical, high altitudes of the Philippines, which are quite different from the dry plains of Northern Ghana.

The error associate with the H_2S test was calculated to be low, 22.22 percent, when testing improved sources and extremely low, 5.88 percent, when testing unimproved sources. Both results indicate that the use of the test improves the predictive ability with regard to the quality of the water. The proportional reduction in error (PRE) was also high for both improved and unimproved sources, 73.74 percent and 54.54 percent respectively, demonstrating an improved predictive ability by using the H_2S test.

Table 14. Error Calculation, H_2S Test

	Improved Sources			**Unimproved Sources**		
	Error	PRE	n	Error	PRE	n
H_2S Test	22.22%	73.74%	26	5.88%	54.54%	85

Overall, given the general statistical results, PRE and p-value, the H_2S test appears to be an appropriate presence/absence test for microbial water quality in improved, and especially unimproved, sources.

7.3 Easygel® Results

Easygel® was used to test 49 unique water samples from the greater Tamale region. Water samples were collected from the same locations as the first 49 H_2S collection sites, however, Easygel® sample size was limited due to the quantity of supplies available in Ghana.

Like that of the H_2S test, the contingency table comparing Easygel® and Quanti-Tray® indicates that the chi-squared test is not appropriate in this case. Instead Fisher's exact test, with general statistical methods, was used to analyze the Easygel® results.

Table 15. Contingency Table, Easygel® vs. Quanti-Tray®

		Quanti-Tray®	
		Presence	Absence
Easygel® Test	Presence	39	1
	Absence	4	5

Like, H_2S, Easygel® proved to be a highly accurate indicator for the presence of *E. Coli*. Easygel® tests produced very high true results, as well as low occurrences of both false positives and false negatives.

The lower negative predictive value indicates that a negative test result will, at times, not correlate with the absence of a contaminant, in this case *E. coli*. Alternatively, high sensitivity and positive predictive values indicate that a positive result will almost always correlate with the result of the standard method and the presence of *E. coli.*

Fisher's exact probability indicated that these results were extremely statistically significant with a p-value of 0.004.

Table 16. Statistical Results, Easygel® vs. Quanti-Tray®

True Result	90%	Sensitivity	91%	Positive Predictive Value	98%
False Positive	2%	Specificity	83%	Negative Predictive Value	56%
False Negative	8%	Error	10%		

Easygel® vs. Quanti-Tray®
Fisher's Exact Probability: 0.0004
Extremely Statistically Significant

The proportional reduction in error for the improved and unimproved sources is shown in Table 17. In the case of improved water sources, adding additional information from the Easygel® test improves the ability to predict if there is fecal contamination present by 50 percent over the assumption that the source is safe. However, for unimproved sources the Easygel® test does not perform well. With a PRE of -100, the assumption that an unimproved source is contaminated better predicts the *E. coli* levels in a sample than does the added results of the Easygel® test.

Table 17. Error Calculation, Easygel® Test

	Improved Sources (%)			**Unimproved Sources (%)**		
	Error	PRE	n	Error	PRE	n
Easygel® Test	50.0	50.00	12	27.02	-100.00	38

While results indicate that the Easygel® test is effective for evaluating improved drinking water sources, with a sample size of only 12 sites, further testing is needed to verify these outcomes.

7.4 Combination Test Results; H_2S + Easygel®

As was the case with the EC-kit, before a contingency table can be created it is necessary to align test results with WHO Risk Levels. A 3x3 contingency matrix was constructed for the performance assessment of the 20ml H_2S test used in conjunction with Easygel® to the results achieved with Quanti-Tray® (The World Health Organization 2006).

Table 18. Contingency Matrix, 20ml H_2S + Easygel® vs. Quanti-Tray®

		Quanti-Tray®		
		Conformity/Low	Intermediate	High/Very High
H_2S +Easygel®	Conformity/Low	8	1	0
	Intermediate	0	7	4
	High/Very High	0	2	27

Using the statistical analysis methods, the above results were produced. The gray shaded section of the contingency table, Table 18, indicates a situation in which the H_2S+Easygel® either over-predicted or accurately predicted the level of risk of a water source. This estimate is included in the results as the conservative estimate.

Table 19. Statistical Results, 20ml H_2S + Easygel® vs. Quanti-Tray®

TR	86%	Sensitivity*	Conformity/Low	100%	PPV*	89%
TR (Cautious)	90%		Intermediate	70%		66%
Error	10%		High/Very High	87%		93%

*Indicated per WHO risk level

Overall, when used in conjunction the two combined tests, H_2S and Easygel®, produce a slightly lower true value than the tests used individually. The pair also appears to perform better in situations of extremely high or low contamination and tends to be less accurate for moderately contaminated water samples, not unlike the EC-Kit.

In examining the error and PRE of the H_2S and Easygel® Test combination, the results indicate that, for improved sources, the method drastically improves the ability to predict the safety of drinking water. For unimproved sources the combination produced smaller, though still positive, standard and conservative PRE values.

Table 20. PRE for the H2S + Easygel® Test Combination

	Improved Sources					Unimproved Sources				
	Error	C. Error	PRE	C. PRE	n	Error	C. Error	PRE	C. PRE	n
H_2S +Easygel®	58.33	58.33	100	100.0	12	40.54	10.81	11.76	0.0	37

*C. is an abbreviation for "conservative"

Overall, while the H_2S + Easygel® Test Combination does improve the predictive ability over the baseline assumptions for microbial water quality, however, in resource limited environments, using the H_2S test as a single presence/absence indicator, may meet needs equally well.

7.5 Colilert Results

Before coupling Colilert with Petrifilm™, as is done in the EC-Kit, it is relevant to compare the individual tests with the Quanti-Tray® as well as to assess their individual performance given improved and unimproved water sources.

Table 21. Contingency Table, Colilert vs. Quanti-Tray®

		Quanti-Tray®	
		Presence	Absence
Colilert	Presence	86	0
	Absence	15	10

Using the result cells from the 2 by 2 contingency table above, general statistical results were calculated in addition to the corresponding p-value.

The Colilert test performed well as compared with Quanti-Tray®, producing a 86 percent true result value with low overall error. The Colilert test also appears to be a particularly good indicator of the presence of contamination with sensitivity and positive predictive values all over 85 percent. The negative predictive value was very low, indicating that a negative Colilert test does not correspond with the absence of *E. coli* 60 percent of the time. This is lower than the value of other tests and warrants further investigation.

Table 22. Statistical Results, Colilert vs. Quanti-Tray®

True Result	86%	Sensitivity	85%	Positive Predictive Value	100%
False Positive	0%	Specificity	100%	Negative Predictive Value	40%
False Negative	13.5%	Error	14%		

In examining the PRE associated with the 10ml pre-dispensed Colilert test there is a clear distinction in its performance between improved and unimproved sources. For improved sources, Colilert clearly provides an improved predictability for microbial water quality with a PRE of 54.54 percent. For unimproved sources, the Colilert test decreases the predictability of water safety and therefore it is more accurate to assume that all unimproved sources are contaminated.

Table 23. PRE for the Colilert Test

	Improved Sources(%)			Unimproved Sources(%)		
	Error	PRE	n	Error	λ	n
Colilert	38.46	54.54	26	16.47	-27.27	85

7.6 Petrifilm™ Results

Like Colilert, the Petrifilm™ test was used to test a total of 111 water samples from the greater Tamale area during January 2011. The 2 by 2 contingency table for the comparison of Petrifilm™ with Quanti-Tray® is shown below.

Table 24. Contingency Table, Petrifilm™ vs. Quanti-Tray®

		Quanti-Tray®	
		Presence	Absence
Petrifilm™	Presence	64	0
	Absence	32	15

Using the information provided by the contingency table statistical results were generated and are presented in Table 25. Results for the Petrifilm™ were mixed. While false positives were low, 0 percent, true results were also on the low side, 71 percent, as compared with other tests in this study. False negatives were on the high side, at 29 percent, dramatically overestimating the safety of the drinking water as indicated by standard methods.

Positive predictive value was very high, 100 percent, indicating that a positive Petrifilm™ test is synonymous the presence of *E. coli* contamination. On the other hand, the low negative predictive value of 32 percent indicates that, a negative result my not be indicative of the absence of *E. coli* in the sample.

Table 25. Statistical Results, Petrifilm™ vs. Quanti-Tray®

True Result	71%	Sensitivity	67%	Positive Predictive Value	100%
False Positive	0%	Specificity	100%	Negative Predictive Value	32%
False Negative	29%	Error	29%		

From the results of the PRE calculations, presented in Table 26, Petrifilm™ on its own appears to be appropriate to test improved, but not unimproved, water sources. This has been the case in a number of the microbial tests in this study and case could be made that unimproved sources in

Northern Ghana are so highly contaminated that they should assumed to always be unsafe for consumption. As compared with other individual tests, the Petrifilm™ does not appear to be the best indicator of microbial contamination on its own in the conditions of this experiment.

Table 26. PRE for the Petrifilm™ Test

	Improved Sources			**Unimproved Sources**		
	Error	PRE	n	Error	PRE	n
Petrifilm™	42.31	48.07	26	16.68	-28.78	85

7.7 Combination Test Results; EC-Kit, Colilert +Petrifilm™

It has been demonstrated in the literature that when used together, the presence/absence 10 ml pre-dispensed Colilert test, and enumerative 1ml Petrifilm™ test, can produce highly accurate results for both improved and unimproved sources as compared with Quanti-Tray® (Chuang 2010) (Trottier, 2010).

Using the WHO risk level categorization, a corresponding 3x3 contingency matrix was created in order to calculate the relevant statistical results for the EC-Kit vs. Quanti-Tray® scenario. The conservative estimate result values are shaded gray in 27. A conservative estimation method, meaning that the EC-Kit indicates the appropriate WHO risk level or higher, essentially taking a precautionary approach to water testing.

Table 27. Contingency Matrix, EC-Kit vs. Quanti-Tray®

		Quanti-Tray®		
		Conformity/Low	Intermediate	High/Very High
EC-Kit	Conformity/Low	21	2	1
	Intermediate	1	17	4
	High/Very High	0	10	54

Using the 3 by 3 contingency matrix, general statistical results were calculated. A summary of the relevant values is provided in Table 28.

The EC-Kit produces approximately the same true value percentage as either of the tests used individually. However, when using conservative result values, the true result percentage jumps to 94 percent. With test results spread across risk levels, sensitivity and positive predictive value results indicate the EC-Kit performs best at either extremely low or extremely high levels of contamination.

Table 28. Statistical Results, EC-Kit vs. Quanti-Tray®

TR	84%	Sensitivity*	Conformity/Low	95%	PPV*	88%
TR (Cautious)	94%		Intermediate	59%		77%
Error	18%		High/Very High	91%		84%

*Indicated per WHO risk level

From this information it is clear that in environmental situations where a distinction must be made between low to moderate levels of contamination the EC-kit is not ideal, however for most rural settings the EC-Kit results can be trusted with a relatively high degree of confidence.

Table 29. PRE for the EC-Kit

	Improved Sources					**Unimproved Sources**				
	Error	C. Error	PRE	C. PRE	n	Error	C. Error	PRE	C. PRE	n
EC-Kit	50.00	30.76	35.00	60.00	26	34.11	11.76	29.26	-	85

*C. is an abbreviation for "conservative"

The EC-Kit, both with normal and conservative estimates, achieved positive PRE values across almost all water sources. For unimproved sources, the conservative interpretation of results is in fact equal to the base assumption that all sources are contaminated. Overall, using the EC-Kit to test any water source will provide an improved predictive value, over baseline assumptions, as to the microbial quality of the water.

8. Field Interview Results

In order to analyze responses to the field interviews, notes from were entered into a Google form and converted to a spreadsheet format. Though this methodology does require the standardization of responses and therefore limits, to come degree, the level of detail that can be recorded, it allows broad trends to be derived from the data. These trends provide insight into the daily practices related to water sources, storage, treatment and health in the greater Tamale area.

8.1 Household Demographics

All villages that were visited during the interview process were governed by a traditional structure in that they were led by one male Chief, who was advised by several males in the village.

Households consisted of several huts clustered around an enclosed central space where most of the cooking, working and socializing took place. It is common for extended families to live together in these compounds in order to share the responsibility for household tasks including gathering water, cleaning clothes, cooking and raising the children.

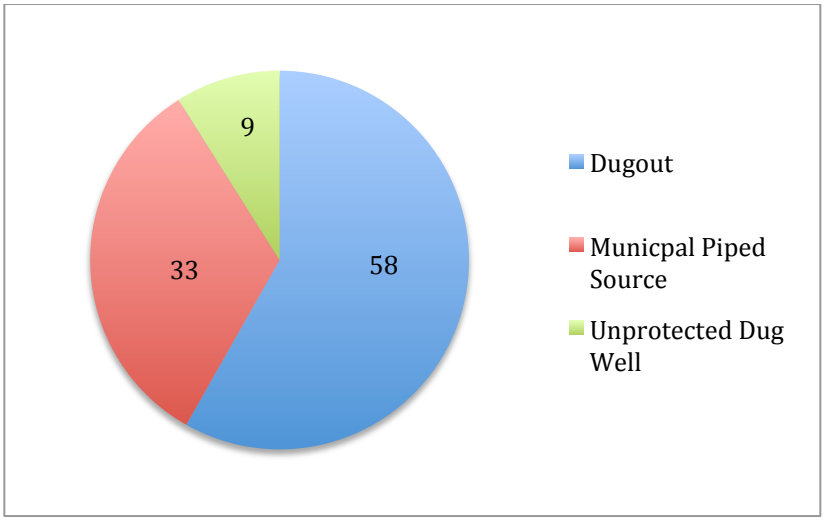
Of the 67 interviews completed, 65 of the respondents was the female of the household. In some cases, several of the women in a compound would collectively answer questions, at times providing conflicting answers.

8.2 Drinking Water Sources and Storage

8.2.1 Sources

The majority of households interviewed, 58 percent, cited a dugout as their primary collection source for drinking water. Municipal piped water was also a not an uncommon source of drinking water, however, in many of these cases pipes were noted to be highly unreliable and a nearby dugout provided "backup" water, anywhere from one to five times per week.

Figure 15. Study Results, Drinking Water Sources (Percent)



Several households paid for access to a piped source. This practice was common if the pipe spigot was within the property of a single household, usually the Chief's. Payment was perceived to be reasonable at either 0.05 Cedi (0.03 USD) per 20-liter jerry can or a similar nominal fee, for example 20 Cedi per month.

Households tended to prefer piped sources due to the low observed turbidity, however, given its unreliability, this was not a feasible option for many. In cases where both dugout and piped sources were available, children were generally discouraged from drinking dugout water. Despite these efforts, it was common to observe children drinking directing from dugouts while their were collecting water or during play.

8.2.2 Storage

Ninety-eight percent of households utilized a traditional ceramic pot container to meet their water storage needs. These pots were embedded in the ground and with dimensions of approximate 40 inches in height with an opening diameter of 10-12 inches. Ceramic pots are preferred to plastic containers due to their ability to keep the water at a relatively cool temperature.

There are challenges with using these traditional containers in that they are open to air, allowing potential contaminants to fall into the drinking water, have no spigot making collecting water nearly impossible without contacting the rest of the stored water and they are deep, making cleaning the lower sections can be problematic.

Figure 16. Water Collection and Storage Containers

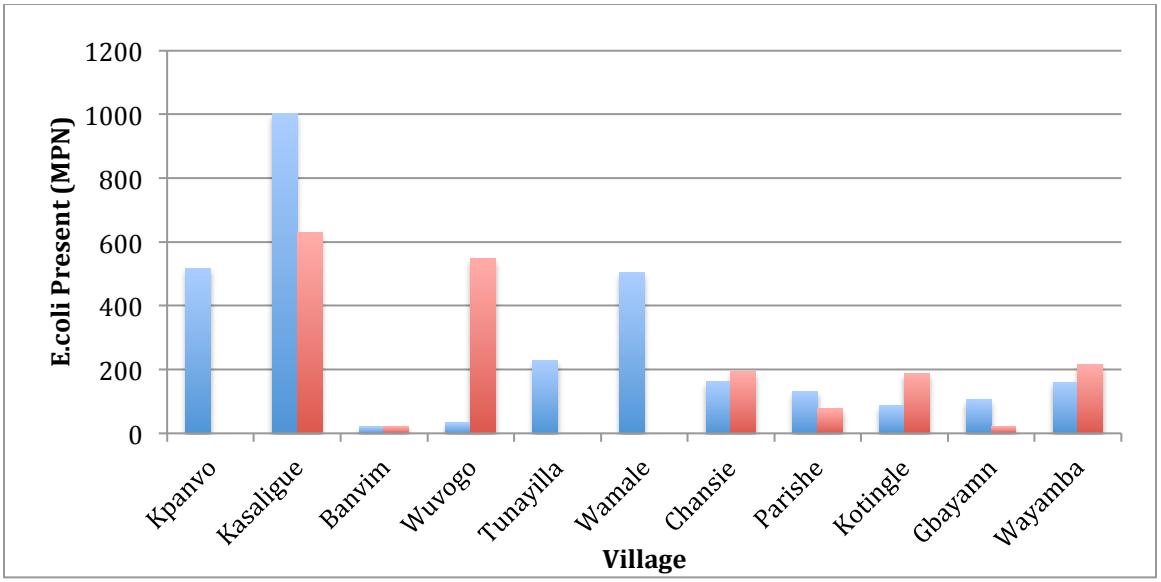
(Samantha O'Keefe, 2011)

8.2.3 The Chief's Exception

With the exception of Wuvogo, the homes of village Chiefs', and their families tended to have equal or slightly better water quality than the rest of village. Outside of those households with a connection to the municipal piped water, it could not be determined specifically what the cause for this trend of improved water quality. The Chief's household, as was the case during this study's household interviews, often has the most interaction with NGOs or other organizations working to improve regional water quality and sanitation practices. Accordingly, the Chief's

household may have the best understanding of water quality and its relationship with disease transmission. Finally, the Chief has more financial resources and therefore may have access to better treatment methods, safer storage, or protected sources.

Figure 17. Chief Household Water Quality vs. Village Water Quality



8.3 Household Water Treatment

8.3.1 Filtration

Of those who reported using a filter, 91 percent use a Guinea Worm cloth filter, making it by far the most common form of treatment employed in rural households. All of these filters were said to have been obtained from an NGO several years ago, which coincides with the timing of the efforts of the Carter Center's Guinea Worm Eradication Program. Starting in 1986, the Center began working the Eradication Campaign, with Ghana as one of the first partner countries. In 2010 transmission of the disease was reported to have stopped, due in large part to the widespread dissemination and use of the simple filters (The Carter Center, 2011). Despite the age and wear of the filters, households still believe they help to improve the quality of their drinking water.

The remainder of household filtration practices, nine percent, were completed using a common household cloth in order to remove major particulates. Depending on the fineness of the stitching, many of these household items have the potential to achieve the same removal levels as an official Guinea Worm filter.

In the villages interviewed there were only two reports of other filter types, one biosand and one ceramic, being used. Both were, at the time of observation, not in use and were instead filled with common household items.

8.3.2 Chemical Treatment

Other forms of drinking water treatment, such as the use of chemical coagulant or disinfectants were significantly less common than filtration. Of the 58 households responding, 38, or 66 percent, reported no water treatment methods were practiced. The remaining households all reported purchasing alum, a common chemical flocculent, and occasionally Aquatabs for personal use.

However, even in households where alum was purchased, its use was not consistent. Only near the end of the dry season when dugouts run low, and thus the water quality is visibly poorer, do households report using additional treatment methods such as alum.

Outside of cost, there are several deterrents to using alum, chlorine or Aquatabs. First, all of these methods alter the flavor of the water. Questions regarding flavor were not specifically asked in household interviews, however prior studies have found aesthetics including flavor, color and temperature, to be of utmost importance when water treatment and consumption decisions even in areas where quality of the water is a major concern.

Additionally, alum and chlorine dosing can be difficult in a traditional pot where the exact volume is unknown. Instead of using standard dosing procedures, a ball of alum is purchased and swirled around in the water source until treatment is deemed complete. The amount of alum added is difficult to estimate, as exposure time and speed of mixing are both arbitrary values that vary household to household. Respondents reported that in some cases, using alum had lead to stomach problems, including diarrhea and fever. These are some of the same health problems that stem from microbially contaminated water sources. Unfortunately, negative health reactions to treatment methods, despite being due to incorrect dosing, only serve to decrease the likelihood that a household will treat its in the future.

8.4 Health and Sanitation Observations

In the rainy season, human feces from open defecation are more likely to be washed in to surface water sources. By containing waste, and separating it from ground water sources, latrines would decrease the occurrence of microbial contamination diseases.

Seventy-seven percent of households interviewed did not have access to a latrine, either in their compound or nearby in their village. Latrines were almost exclusively provided at little to no cost to communities by an outside NGO. There was no mention or knowledge of government sponsored sanitation efforts.

Almost all of respondents articulated their preference for latrine use to open defecation. Latrine preference was due to an increased perception of privacy and safety, from the elements, as well as from animals. However, of the 23 percent that did have access to a latrine, only 66 percent of

these reported using the latrine on a regular basis despite the fact that almost 100 percent of respondents reporting they preferred them to the bush.

During a visit to the Shekhinah Clinic near Wamale, an interview was conducted with Dr. Kwame. From his observations, the majority of childhood illness seems to come during the rainy season. Common ailments he treats were diarrhea, malaria or general fever and achiness. It is disappointing to see as many of the diseases he treats are, in his words. “preventable” (Kwame, 2011).

9. Issues of Science Policy in Water Quality Testing

Policy makers face very different pressures and expectations than those of the scientific community. They are often expected by the public to act quickly and cost-efficiently to solve a problem at hand. The science community, however, places value in creating a more complete body of knowledge, as a whole, and encourages experimentation in order to arrival at an eventual truth. The fundamental difference in time scales within which the players operate can make communication challenging.

Dr. Tikki Pang, director of the research and cooperation department of the WHO, points out that scientific research "only provides one type of evidence." Policy decisions, unlike scientific experiments, are made with pulls from political and economic factors and considerations surrounding social and culture contexts (Pang 2007). He goes further to eloquently summarize the three major questions policy makers in the public health world ask themselves when faced with promoting a new technology that may not be fully vetted, like the H_2S test. "Can it work? Will it work? Is it worth it?" (Pang, 2007).

In the case of the H_2S test, these three basic questions can be translated into the following: Is hydrogen sulfide producing bacteria a valid indicator of unsafe drinking water? In what conditions does the H_2S test work and how well? And, is promoting the new method, given the potential risks, worth it? With each of these questions come concerns from both the policy and scientific perspectives that must be considered before moving forward with the H_2S test.

9.1 Can the H_2S Test Work?

From the literature review presented in Chapter 4, numerous studies indicate that the detection of H_2S in a water source has been shown to correlate with the presence of *E. coli,* as indicated by standard microbial testing methods. Reasonable results were attained using several varieties of substrate compositions, as well as across a range of environmental climates including Chile, India, Indonesia and the Philippines. Many of the studies specifically recommended the H_2S test for field-based work where rapid assessment of a high number of water sources had to be assessed for safety (Grant Z. , 1996) (J. Pillai, 1999). From both a science and policy viewpoint, the H_2S test has the potential to identify microbially safe and unsafe drinking water sources.

9.2 Will the H_2S Test Work?

This question highlights the difference in the way the two communities define "work" when referring to the performance of a microbial indicator.

For the international and development communities, the purpose of the H_2S test is provide a lower-cost, easy to use tests that will allow for more frequent microbial water testing. In this scenario, for a technology to "work," reasonable accuracy is required, however given the importance of other criteria; the community accepts a tradeoff between accuracy and price. Given

the importance of clean water to global health, a highly usable test is almost as, if not more, helpful as a test that is 100 percent accurate.

Scientists, Dr. Mark Sobsey of the University of North Carolina among them, give pause when asked if the H_2S test "works." From a scientific perspective, the test has not been rigorously vetted as the studies that have been performed vary in field collection methodology, substrate composition, media form (liquid, paper strip etc.) and standard methods used to evaluate performance (Sobsey, 2004). The majority of criteria required for determining an ideal indicator have not been tested for H_2S and many scientists feel it is premature to promote the test on the basis of positive field tests that indicate a correlation with the presence of other fecal indicators.

9.3 Performance of the H_2S Test

In assessing the performance of microbial water quality tests, there is the potential for two distinct types of errors, each with their own pros and cons. False negatives, occur when standard methods indicate the presence of contamination, a positive result, but the "New Test" indicates that the water is safe, a negative result. Alternatively, false positives, occur when a "New Test" indicates that a water source is contaminated, a positive result, when in fact it is not. In practice, this means rejecting or limiting the use of a safe water source. The figure below presents a contingency table indicating which result cells correspond to incorrect assumptions with regard to the water safety.

Figure 18. False Negative vs. False Positives

		Standard Method	
		Presence	**Absence**
New Test	**Presence**	TR	FP
	Absence	FN	TR

Though neither error is ideal, depending on the context of a situation, either false negatives or false positives may be preferable. For example, in situations where there is extreme water scarcity and daily drinking water supply is very low, false positives leading to the incorrect rejection of a source could have highly detrimental affects on the health of a community, and thus false negatives would possibly create less risk. Conversely, in a region where there is ample supply of freshwater sources but perhaps, a highly vulnerable population, it may be in the best interest in the community to be more cautious in terms of water quality requirements.

It is with these tradeoffs in mind that policy makers attempt to recommend specific microbial tests for general use. In some cases, the performance of a test may vary significantly across geographic regions or climate zones making it difficult to identify a single method or methodology that is universally appropriate.

9.4 Promotion of the H_2S Test

Similar to the example shown in Figure 18, the decision by development agencies to promote the H_2S test as acceptably accurate can also be broken table into two distinct options: Correct (Type 1) or Erroneous (Type 2). In this case, Type 1 errors refer to the opportunity lost from not approving a helpful technology immediately (Oye K. , 2010).

Figure 19. Type 1 vs. Type 2 Errors

	H_2S Test produces accurate results	**H_2S Test does not produce accurate results**
Accept	Correct Policy Decision	Type 2 Error
Reject	Type 1 Error	Correct Policy Decision

Type 2 errors refer to an acceptance of risk of potentially inaccurate results, which could lead to unsafe water being consumed. While large industrialized countries have the ability to use the most rigorously tested, and expensive methods, of water testing, not all nations have this luxury.

Developing countries often rely on NGOs or large international agencies to monitor the quality of their water sources. The testing they are able to complete, given their human and capital resource constraints, is minimal at best, and thus the true safety of drinking water in the country is unknown. The proliferation of the H_2S test appears to be worth the risk of a small percentage of inaccurate results in order to achieve a significant reduction in water related diseases and deaths.

The precautionary principle, when applied to water testing, advises heavily to the acceptance of Type 1 errors until further verification can be done to confirm the legitimacy of the test. In many cases, this principle is also the approach of the scientific community in order to protect its integrity in the eye of the public (Oye K. , 2010). There will never be an indicator organism that accurately identifies safe and unsafe drinking water 100 percent of the time.

10. Community Water Supply in Ghana

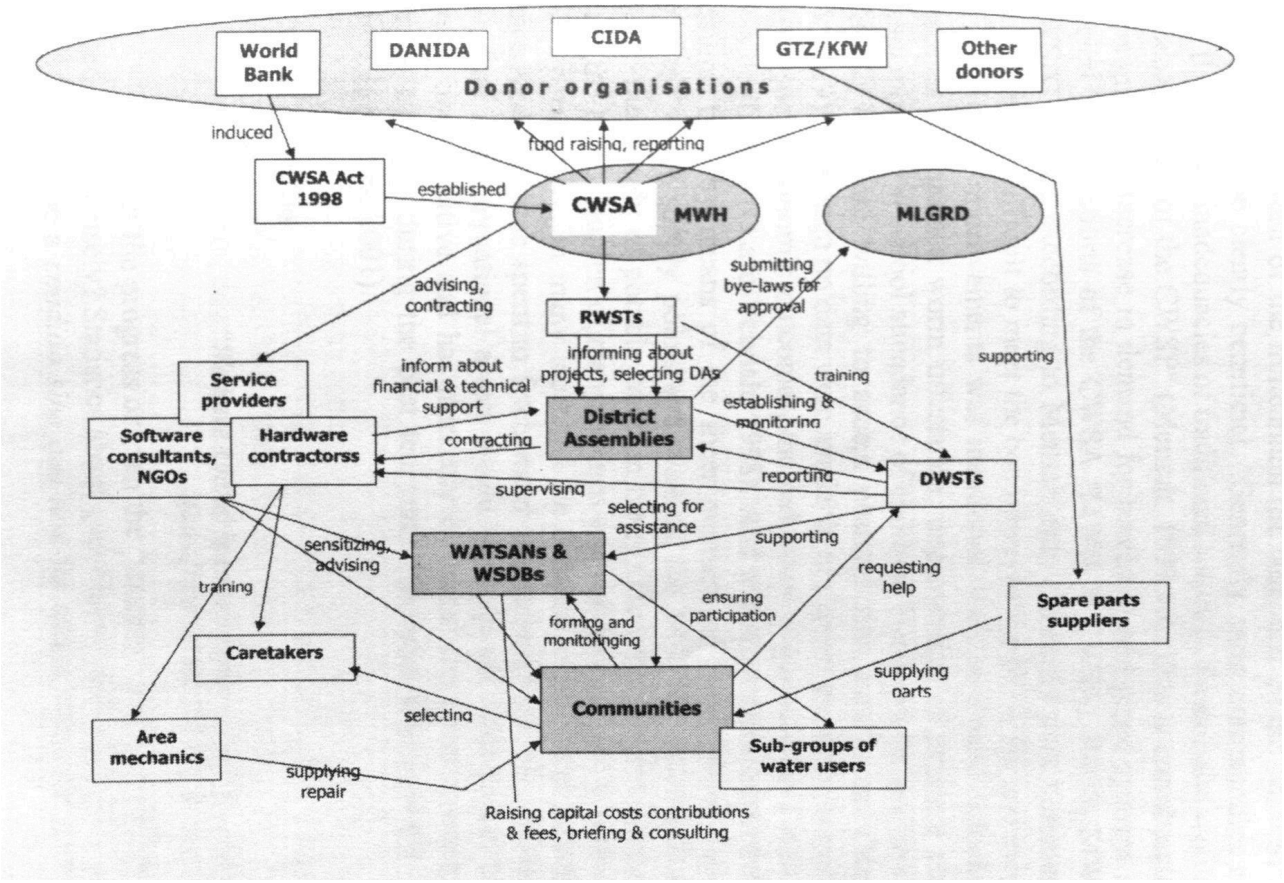
Given the poor coverage of improved water sources in the Northern Region, an investigation and evaluation of the regulatory bodies, specifically the Community Water and Sanitation Agency (CWSA), and other lesser players, is warranted. Based on findings in the literature and observations, recommendations for potential improvements will be made in order to increase the percentage of Ghanaian's in the Northern Region with access to potable water.

10.1 Water Regulation in Ghana

In the latter half of the 20th century, the development community saw the failure of the supply-driven utility model, and experienced a strong shift to more demand-driven approach. Placing an emphasis on the demand side, agencies sought to more actively engage communities in developing their own resources so that they would take ownership of infrastructure projects. Ghana was no exception and over the past 20 years has reorganized the water sector to become regulated via a highly decentralized model (Fuest, Demand-oriented Community Water Supply in Ghana, 2006).

Today, individual communities communicate their needs to District Assemblies (DA), which, in turn, coordinate with the region office of the CWSA. Region offices receive funding from a central Ministry of the Government. Though a somewhat involved process, as diagramed in Figure 20, decision-making is left in the hands of the individual communities and the regional DAs, emphasizing the local ownership of water projects.

Figure 20. A map of regulatory interactions, Water Sector, Ghana



(Fuest, Demand-oriented Community Water Supply in Ghana, 2006)

10.2 External Stakeholders

The following stakeholders are institutions that, either formally or informally, play a role in the supply, distribution and overall management of the water sector in Ghana. The stakeholders listed here are in no way an exhaustive list, however, they do represent the largest and most relevant players involved.

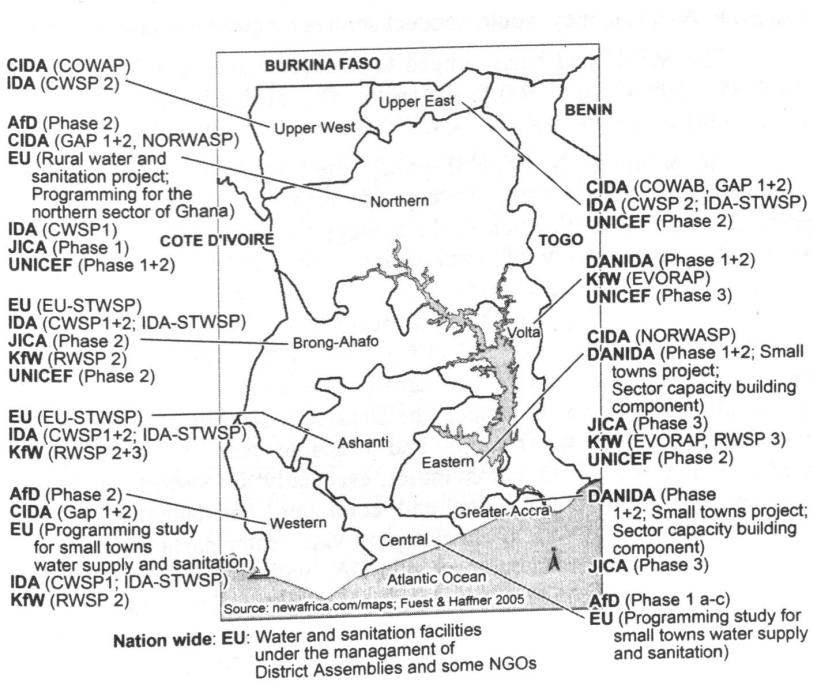
10.2.1 International Actors

Due in large part to the stability of the Ghanaian Government as an institution and a wealth of natural resources in the country, including gold and other minerals, high levels of international aid has entered the country. Many international agencies have also created permanent establishments in the more industrial regions of the country, including Accra and Kumasi.

Arguably the most involved international group, specifically within the water sector, is the World Bank. Since the early 1990s, the Bank has been heavily involved in the restructuring of Ghana's water management framework and lobbied heavily for a decentralized approach from which the CWSA was formed.

With the Bank are a number of other active donor agencies including DANIDA, the Danish International Development Agency, CIDA, the Canadian International Development Agency and KfW, the German International Development Agency. Figure 21 provides a glimpse of the involvement of international donors in the country in 2006. As is clearly shown, multiple projects in each region of the country were simultaneously ongoing. What is most striking, perhaps, is that the image only documents those efforts in the WASH sector. Involvement in the country as a whole, was, and still is, much more prevalent than Figure 21 depicts.

Figure 21. International WASH Involvement, Ghana



(Fuest, Demand-oriented Community Water Supply in Ghana, 2006)

While the nature of each investment is unique, many projects funded by outside agencies, are done so via a loan. Over time, Ghana, unable to pay off its loans with revenues generated in country, has become essentially dependent on foreign aid and loan forgiveness to sustain itself (Meng, 2004).

10.2.2 Non-Governmental Organizations (NGOs)

The impact of NGOs in Ghana is large due to their extreme prevalence across all regions of the country. In 1996, it was estimated that approximate 320 foreign and local NGOs were operational. That number has grown rapidly to between 900 and 1500 unique organizations in 2011 (Bob-Milliar, 2011).

Due to the particularly high activity of NGOs in the water and sanitation sector, in 2001 an association, the Coalition of NGOs in the Water and Sanitation Sector, was established to coordinate the entities to minimize duplication. More recently, the Inter-Agency Coordinating Committee (ICC), originally focused on coordinating Guinea Worm eradication efforts, has become the coordinating body among NGOs. However, with this number of autonomous agencies existing in Ghana, it is often difficult to monitor their activity to ensure that efforts are in line with national goals and not duplicated across organizations.

10.3 Internal Stakeholders

10.3.1 The Government of Ghana

The Ministry of Water Resources, Works and Housing (MWRWH) is the regulatory body within the Ghanaian Government charged the oversight of water supply and sanitation. The main objectives of the Ministry are (Ghana-net, 2001):

1. Policy Planning Budgeting Monitoring and Evaluation;
2. Human Resource Development;
3. Research Statistics Information Management; and
4. Administration and Finance.

Under the MWRWH there are two separate bodies, The Ghana Water Company and the Community Water and Sanitation Agency (CWSA) tasked with providing water to urban and rural populations respectively. Given the rural nature of this study, further exploration of the CWSA's institutional structure and practices is presented below.

The CWSA is tasked by the Government of Ghana to implement The National Community Water And Sanitation Programme, which set a target of 76 percent water and sanitation coverage in rural Ghana by 2015 (Community Water and Sanitation Agency, 2009).

10.4 The Community Water and Sanitation Agency (CWSA)

10.4.1 Founding Mandate

In 1998, the Community Water and Sanitation Agency became a fully autonomous entity and was mandated by law to enable the full implementation of the National Community Water and Sanitation Program (NCWSP) (Water and Sanitation Program, 2002).

The CWSA Act of 1998 consists of two main parts; Part 1 establishes the agency, outlines its structure, functions, main members and regional offices, while Part 2 discusses administrative, financial and general provisions (CWSAGH, 1998). The CWSA has three main objective areas; safe water supply; improved sanitation. This analysis will only address the quality and consistency of the water supply.

The major tasks of the CWSA include coordinating and facilitating the construction and maintenance of water related infrastructure between the public sector and private sector, as well as the NGOs. The CWSA is limited to one regional office in each of Ghana's regions, and a total staff of under 200 persons. (CWSAGH, 1998) The CWSA was also designed as a demand-driven entity for villages of less than 50,000 persons, meaning that a District Assembly must seek out the CWSA and request service.

Since it's inception, the CWSA has worked in unconventional ways with existing NGOs in Ghana. In the past, the regional government would hire individual community development

workers and pay them to facilitate community involvement in projects. Instead, the CWSA has begun to contract organizations already working in rural areas to complete these tasks (Community Water and Sanitation Agency, 2009). Unfortunately, NGOs, as autonomous players, often operate according to their own goals and agendas that often conflict with those established by the Ghanaian Government and water authorities, making collaborations more challenging.

10.4.2 Public Awareness

There has been no formal polling conducted with regards to the performance to the CWSA, however, informal interviewing was done indicated that much of the rural community is not aware of the existence of the CWSA. This lack of information and communication significantly limits the ability of the agency to carry out its job (United Nations, 2004). Informally interviewing also indicated that most villagers are "surprised" when their pipe turns on.

District Assemblies

District Assemblies (DAs) are local governing authorities made up of approximately 70 percent elected, and 30 percent appointed, representatives. DAs are highly integral bodies in terms of rural water supply as they have the authority and responsibility for district level supply procurement. Following the demand-driven model previously discussed, the DAs collect and prioritize demands for service from villages within their district and coordinate with the CWSA to initiate projects when funding is available. The DAs also act as owner for any systems that are installed, and maintain and monitor those systems, as well as facilitating the collection and management of community tariffs.

Communities, or villages, were ideally to voice their concerns and needs to the DAs who would in turn involve the communities in decision-making processes including pipe locations, etc.

10.4.3 Disparities in Performance

The CWSA performance was measured internally by several quantitative metrics, including the new facilities, or prior facilities repaired, and overall access to improved drinking water sources.

In terms of physical infrastructure improvements, the chart below, adapted from the 2003 unpublished progress report on the CWSA, appears to demonstrate the rapid scale achieved by the Agency in just nine years (MWH & CWSA, 2003).

Figure 22. Facility Creation/Repair CWSA, 1994-2003

Supply Type	Number Achieved
Boreholes	5410
Hand-dug wells	1259
Pipe systems	453
Repair of boreholes	3205
Repair of hand-dug wells	72
Repair of pipe systems	4189

With regard to access to improved, or safe, drinking water sources, the CWSA also appears to have performed well according to international agency metrics. In 2003 the World Bank reported that over 85 percent of the poor and 96 percent of the non-poor had access to improved drinking water (World Bank Group, 2003).

These results, however, starkly contradict data gathered by this study. In the greater Tamale region it was determined that 67 percent of interview respondents relied on an unimproved supply for their primary drinking water source. For those households who did have access to an improved source, it is estimated, based on microbial testing, that forty-five percent have unsafe levels of contamination. This distinction made between poor and non-poor households by the World Bank Group was not done in this study, though based on the heavily rural sampling bias, it is assuming the vast majority of interview respondents were poor.

In reality, given the mobility of the Ghanaian population, inaccurate data collection, and recording procedures, it is a difficult task to determine the actual performance of the CWSA.

10.5 Challenges in Water Governance

10.5.1 Impact of International Involvement

The involvement of these actors was omnipresent, with involvement of one or more agency in each of Ghana's ten regions. Though well intentioned, the levels of international aid have created what some have called a "risk-averse lethargy." Due to the intimate involvement of outsiders, Ghanaians, and in this case their government institutions, have not sought to gain the skills need to manage large infrastructure projects (Agrawal, 2008).

The World Bank's own "Country Assistance Review" identified the need to focus in the next ten years on "promoting institutional development and capacity building" (World Bank Group, 2011).

Lastly, the presence of international organizations within a developing country has the potential to lead to an "intranational brain drain," where the most educated citizens who choose to stay in country after completing their education, choose to work for international agencies with higher pay rather than their local government. Due to the high volume of both international donors and NGOs, the influence of these organizations is strongly felt in both the urban and rural areas of the country.

10.5.2 Human Capacity and Resource Issues

In the early 2000s, it became apparent that the CWSA lacked manpower, with reports siting an inability to fill positions, and a high staff turnover potentially caused by burnout of the existing employees (Community Water and Sanitation Agency, 2009).

Furthermore, there is consistently a lack qualified staff as Ghana, like many developing countries, is a victim of substantial "brain drain" whereby many well-educated individuals leave the country to seek better employment opportunities elsewhere. The staff that is present, plagued by unreliable pay and dissatisfaction for their working environment, is often unmotivated, and work quality and quantity suffers (Ashahid, 2011).

Finally, funding by the central Government of Ghana to the CWSA was reported to be far lower than what was needed given the scope of the undertaking the Agency was assigned (Agrawal, 2008) (Community Water and Sanitation Agency, 2009).

10.5.3 Ambiguity of Mandate

In comparing the CWSA's mandate with the resources with was allocated and the authority it was given, it is not surprising that the Agency has had a difficult time reaching its goals. According to Fuest, this is not an uncommon occurrence where international aid money is involved. Eager for the influx of capital, governments quickly establish national initiatives with goals, timelines, budgets, etc. in order to gain investment. Unfortunately, these promises are left unfulfilled due to the inability of the government to facilitate intermediate steps at a rate fast enough to truly support the activities originally planned (Fuest, Demand-oriented Community Water Supply in Ghana, 2006).

The CWSA also suffers from regulatory constraints in that, with every international donation comes a set of criteria for how and when that money can be spent. Given the number of unique donors and requirements involved with the Government of Ghana, the CWSA is ill equipped allocate and report on their funding use in this type of complex structure.

Finally, in the rural areas the CWSA is, to some degree, competing with NGOs and donor agency that are distributing water or providing taps for free. This disincentivizes communities to activity participate in the dialogue the organization was set up to facilitate (Ashahid, 2011).

11. Conclusions and Recommendations

11.1 Conclusions

1) To confirm the accuracy of the 20ml H_2S tests as a single presence/absence indicator for fecal coliforms.

The H_2S test achieved a p-value of 0.0001, indicating results were extremely statistically significant, and a PRE of 73.74 and 55.54 percent for improved and unimproved sources respectively. Combining the high percent of true value results, improved predictive ability obtained with the test, the ease of use and low cost, the 20 ml H_2S test in an appropriate presence/absence to be used when testing improved and unimproved water sources in the environmental conditions present in Northern Ghana.

2) To establish the accuracy of Easygel® as a single enumerative test for fecal coliform.

Results from the enumerative Easygel® test, were mixed across water source types. For improved sources, Easygel® achieved a PRE of 50, indicating it has a reasonable ability to increase the predictability of the water quality in addition to low incidence of false positives and false negatives. However for unimproved sources, a PRE of -100 was calculated indicating that it is better to assume that an unimproved source is contaminated than perform the test. In addition, Easygel®, while not requiring electricity for incubation, still requires media to be frozen up to two weeks prior to use making it less than ideal is rural areas of developing countries. The test may be appropriate for peri-urban environments where water sources are likely improved and there is potential access to electricity.

Additional testing of Easygel® is recommended as these results were achieved with a small sample size for improved sources and may not be replicable on a larger scale.

3) To verify the accuracy of the 20 ml H_2S test used in combination with Easygel® as an improved method of quantifying contamination as compared with the individual tests.

When examining the use of the 20ml H_2S test with the enumerative Easygel® test it was determined that for improved sources the combination performed well, achieving conservative true result of 90 percent and a PRE of 100.

As was the case with the performance of Easygel® in unimproved sources, in only a small number of cases did the combination of H_2S and Easygel® provide increased accuracy in the identification of safe unimproved water sources. As compared with a conservative assumption that all unimproved water sources were contaminated, the pair offered no improvement in predictive value.

Should the recommended additional testing of Easygel® produce positive results, subsequent testing of the combined H_2S and Easygel® tests is recommended.

4) To further confirm the accuracy of the EC-Kit as an improved method of quantifying contamination as compared with the individual tests.

The EC-Kit, verified for use in the Philippines in 2010 by Chuang et al., also performed well in this study. PRE values were comparable across improved and unimproved water sources and fell between 30 and 35. Though not as large an increase in predictive value as several of the individual tests explored in this study, the EC-Kit proves to be reasonably accurate regardless of the origin of the water source.

5) To use the results of an informal behavioral household interview and performance review of the Community Water and Sanitation Agency (CWSA) to provide context and recommendations for policy action to improve access to potable water in Northern Ghana.

Overall, access to safe drinking water for rural populations in Northern Ghana is minimal. Survey results indicate that the majority of households primarily rely on unprotected and highly contaminated water sources and rarely is water treated more extensively than basic cloth filtration. Furthermore, households who do have access to improved water sources perceive it to be safe, when a substantial portion of the time it is not. Piped sources are unreliable as they are often closed for three to five days a week.

The CWSA is also facing challenges due to the influence and impact of large international agencies and NGOs in Ghana, intranational and international brain drain, and a lack of resources and authority to fulfill their mandate. If Ghana hopes to improve access to safe drinking water across its most rural areas, a reevaluation of the governing policies of the agency is necessary.

11.2 Recommendations for Future Work

1) Verification of Easygel® as a single enumerative test

Easygel® was performed on the smallest sample size (49) used in this study and thus it is recommended that a larger study be undertaken in order to draw conclusive results with respect to its use in determining microbial water quality.

2) Establishment of a MPN H_2S Test

Given the highly accurate performance of the H_2S test with both improved and unimproved water sources, it follows that H_2S producing bacteria could provide an enumerative indication of contamination levels. There is currently a version of an H_2S MPN test available on the market (HACH), however, applicability to a variety of environmental regions has yet to be determined. Also, the development of a low-cost, laboratory made product may be able to decrease the cost per test, and increase ease of test execution for the user.

3) Microbiological verification of H_2S bacteria as indicator organism

In order to verify a single specie or species of hydrogen sulfide producing bacteria as valid indicator organisms, a database analysis approach, utilizing the Human Microbiome Project and Genabank data, is recommended.

Identifying an indicator directly from the human gut would potentially eliminate the ambiguity of current test methods that do not distinguish between contamination from human fecal source and warm-blooded animal fecal sources. Though both have the ability to transmit disease vectors, infection and subsequently disease is more probable, and therefore more dangerous, from a human source. Appendix G contains a full research proposal for this recommendation.

11.3 Policy Recommendations

1) Reevaluation of a Demand Driven Model

In theory, the demand-driven model is ideal as it fully engages communities in solving their own problems, as well as efficiently regulating water supply so that access is only provided where it is needed. In practice, however, Ghana has faced many impediments to the widespread execution of this model. Communication between villages and the District Assemblies (DA) is limited and often the ability to supply water is used as a political asset. In the informal interviews conducted as part of this research, many households were not aware of the existence of the CWSA or their responsibility to request access to water before it can be supplied.

The Government of Ghana should reevaluate the demand driven model of the CWSA to determine if it is, in fact, truly appropriate for their context or if it is a fabrication of donor agencies that is unrealistic in the context of current national resources, specifically with regard to human capital.

2) Regulation of NGOs and International Donors

Ghana has almost more NGOs per capita than any other country in Africa (Moyo, 2009). While these organizations serve a vital purpose in meeting the needs of the Ghanaian population, they also have demonstrated the ability to fundamentally alter the perception of what should and should not be provided for in the water and sanitation sector. Handouts provided by NGOs, though well intentioned, effectively discourage communities from engaging in the participatory demand-driven model proliferated by the Ministry of Water and Housing through the CWSA.

In addition, the acceptance of large sums of foreign aid in the form of loans, has forced Ghana into debt, therefore making the Government dependent on foreign investment for survival. Though sure to be a long-term issue, Ghana must set limits to the amount of foreign aid it accept and work to bolster its own banking system to support internal financial investment.

It is recommended that the Government of Ghana consider more stringent requirements for incorporation of non-profit agencies, including a thorough review of organizational practices and goals to ensure efforts of the NGO will be working constructively with government efforts.

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Appendix A. Contingency Tables

20 ml H_2S Test		Quanti-Tray®	
		Presence	Absence
20 ml H_2S	Presence	85	0
	Absence	11	15

20 ml H_2S Test Improved		Quanti-Tray®	
		Presence	Absence
20 ml H_2S	Presence	16	0
	Absence	6	4

20 ml H_2S Test Unimproved		Quanti-Tray®	
		Presence	Absence
20 ml H_2S Test	Presence	69	0
	Absence	5	11

Easygel® Test		Quanti-Tray®	
		Presence	Absence
Easygel® Test	Presence	39	1
	Absence	4	5

Easygel® Test Improved		Quanti-Tray®	
		Presence	Absence
Easygel® Test	Presence	6	0
	Absence	6	0

Easygel® Unimproved		Quanti-Tray®	
		Presence	Absence
Easygel® Test	Presence	27	6
	Absence	4	0

H_2S +E. gel Cont. Table		Quanti-Tray®			
		Conformity/Low	Intermediate	High/Very High	Total
H_2S +Easygel®	Conformity/Low	8	1	0	9
	Intermediate	0	7	4	11
	High/Very High	0	2	27	29
Total		8	10	31	49

H_2S +E. gel Improved		Quanti-Tray®			
		Conformity/Low	Intermediate	High/Very High	Total
H_2S +Easygel®	Conformity/Low	0	1	1	2
	Intermediate	0	0	5	5
	High/Very High	0	0	5	5
Total					12

H_2S +E.gel Unimproved		Quanti-Tray®			
		Conformity/Low	Intermediate	High/Very High	Total
H_2S +Easygel®	Conformity/Low	0	0	1	1
	Intermediate	8	6	3	17
	High/Very High	0	3	16	19
Total					37

Colilert All		Quanti-Tray®	
		Presence	Absence
Colilert	Presence	86	0
	Absence	15	10

Colilert Improved		Quanti-Tray®	
		Presence	Absence
Colilert	Presence	13	1
	Absence	9	3

Colilert Unimproved		Quanti-Tray®	
		Presence	Absence
Colilert	Presence	66	6
	Absence	8	5

Petrifilm™ All		Quanti-Tray®	
		Presence	Absence
Petrifilm™	Presence	64	0
	Absence	32	15

Petrifilm™ Improved		Quanti-Tray®	
		Presence	Absence
Petrifilm™	Presence	12	1
	Absence	10	3

Petrifilm™ Unimproved		Quanti-Tray®	
		Presence	Absence
Petrifilm™	Presence	47	5
	Absence	27	6

EC-Kit All		Quanti-Tray®			
		Conformity/Low	Intermediate	High/Very High	Total
	Conformity/Low	21	2	1	24
	Intermediate	1	17	4	22
Petrifilm™ +Colilert	High/Very High	0	10	54	64
Total		22	29	59	110

EC-Kit Improved		Quanti-Tray®			
		Conformity/Low	Intermediate	High/Very High	Total
	Conformity/Low	4	1	7	12
	Intermediate	1	1	0	2
Petrifilm™ +Colilert	High/Very High	1	3	8	12
Total		6	5	15	26

EC-Kit Unimproved		Quanti-Tray®			
		Conformity/Low	Intermediate	High/Very High	Total
	Conformity/Low	9	1	3	13
	Intermediate	2	12	6	20
Petrifilm™ +Colilert	High/Very High	6	11	35	52
Total		17	24	44	85

Appendix B. PRE Assumption Tables

H_2S Assumption Table Improved

		Standard	
		Presence	Absence
Prediction	Presence	0	0
	Absence	22	4

H_2S Assumption Table Unimproved

		Standard	
		Presence	Absence
Prediction	Presence	74	11
	Absence	0	0

E.gel Assumption Table Improved

		Standard	
		Presence	Absence
Prediction	Presence	0	0
	Absence	12	0

E. gel Assumption Table Unimproved

		Standard	
		Presence	Absence
Prediction	Presence	33	5
	Absence	0	0

Assumption
H_2S +E.gel

Improved		Standard			
		Conformity/Low	Intermediate	High/Very High	Total
Prediction	Conformity/Low	0	1	11	12
	Intermediate	0	0	0	0
	High/Very High	0	0	0	0
Total					12

Assumption
H_2S +E.gel

Unimproved		Standard			
		Conformity/Low	Intermediate	High/Very High	Total
Prediction	Conformity/Low	0	0	0	0
	Intermediate	0	0	0	0
	High/Very High	8	9	20	37
Total					37

Colil. Assumption Improved

		Standard	
		Presence	Absence
Prediction	Presence	0	0
	Absence	22	4

Colil. Assumption Unimproved

		Standard	
		Presence	Absence
Prediction	Presence	74	11
	Absence	0	0

P.film Assumption Improved

		Standard	
		Presence	Absence
Prediction	Presence	0	0
	Absence	22	4

P.film Assumption Unimproved

		Standard	
		Presence	Absence
Prediction	Presence	74	11
	Absence	0	0

Assumption
EC-Kit Improved

		Standard			
		Conformity/Low	Intermediate	High/Very High	Total
Prediction	Conformity/Low	6	5	15	26
	Intermediate	0	0	0	0
	High/Very High	0	0	0	0
Total					26

Assumption
EC-Kit Unimproved

		Standard			
		Conformity/Low	Intermediate	High/Very High	Total
Prediction	Conformity/Low	0	0	0	0
	Intermediate	0	0	0	0
	High/Very High	17	24	44	85
Total					85

Appendix C. The H_2S Test Procedure

*As prepared by Stephanie Trottier, 2010

The procedure used to prepare the H_2S culture media (M1 and M2), process the samples and interpret the results were taken from (Manja, Maurya, & Rao, 1982); (Grant & Ziel, 1996); (Pillai, Mathew, Gibbs, & Ho, 1999), (IDRC, 1998), and (Venkobachar, Kumar, Talreja, Kumar, & Iyengar, 1994). Furthermore, the originial medium established by (Manja, Maurya, & Rao, 1982)) used 1 mL of Teepol. However, since Teepol is not widely available, (Grant & Ziel, 1996)) used lauryl sulfate salts (or sodium lauryl sulfate) instead. Also, the H_2S test reagent includes sodium thiosulfate, which neutralizes chlorine present in a water sample. This means that the H_2S test is a suitable microbiological test for chlorinated water supplies

H_2S medium

Bacteriological peptone	40.0 g
Dipotassium hydrogen phosphate	3.00 g
Ferric ammonium citrate	1.50 g
Sodium thiosulphate	2.00 g
Teepol 601/Sodium lauryl sulfate	0.20 g
L-cystine (for M2 medium only)	0.25 g
Water, distilled or boiled tap	100.0 mL

Preparation of the H_2S-test reagent

1. Weigh the above listed dry ingredients on a well-calibrated scale.
2. Prepare the 100-mL distilled or boiled water in a 200-mL beaker.
3. Carefully add the dry reagents to the beaker of water, stirring constantly until mixture seems homogeneous.

Preparation of the test tubes and bottles

100-mL and 20-mL samples

1. Any kind of 50- to 200-mL sterilized glass bottles with heat resistant caps, or 4-oz Whirl-Pak bags can be used.
2. Taking Kleenex type paper, or non toxic paper, place a sufficient amount in each container so as to allow the paper to readily absorb 1 mL (for the 20-mL test) or 2.5 mL (for the 100-mL test) of the culture medium. The absorbant paper will be approximately 2 cm x 3 cm to 5 x 5 cm in size.
3. Place the bottles (loosely capped) in an autoclave at 115˚C for 15 minutes. Then place the bottles in a dry hot air oven at 55˚C for 60 minutes to sterilize and dry. Alternatively, the bottes can be placed in a hot air oven at 70˚C for 60 minutes. Cool the bottles until they reach ambient temperature. The media can be stored for up to 6 months in a cool, dry and dark place. The bottles must be opened only immediately before collecting the water sample.

4. If Whirl-Pak bags are used, dry the paper strip media in a hot air oven at 55˚C for 60 minutes. Place the strips in a plastic bag and store in a cool, dry and dark place for up to 6 months. The paper strip should be placed into the Whirl-Pak bag immediately before collecting the water sample.

10-mL sample

1. Use test tubes with heat resistant screw caps.
2. Add 10 mL of water to one tube and using a permanent marking pen, make a mark on the tube at the bottom of the meniscus of the added water. Using this mark as a guide, perpare as many tubes as needed with a 10-mL mark line.
3. Taking Kleenex type paper, or non-toxic paper, place a sufficient amount in each container so as to allow the paper to readily absorb 0.5 mL of the culture medium. The absorbant paper will be approximately 1 cm x 2 cm in size.
4. The tubes can then be loosely capped and autoclaved for 15 minutes at 115˚C. Then place the bottles in a dry hot air oven at 55˚C for 60 minutes to sterilize and dry. Alternatively, the bottes can be placed in a hot air oven at 70˚C for 60 minutes. Cool the bottles until they reach ambient temperature. The media can be stored for up to 6 months in a cool, dry and dark place. The bottles must be opened only immediately before collecting the water sample.

Preservation and incubation of samples

When the samples are collected directly into bottles, sterile sampling bags, or test tubes (with paper strips), these samples must be processed and incubated as soon as possible. In tropical regions, the samples can be incubated at room temperature. Incubation should continue for a maximum of 48 hours and should be interpretated within 24 to 48 hours of incubation.

Interpretation of results

Samples should be checked after 1 hour of incubation to avoid false positives, after which they should be inspected after 24 hours. The test is considered positive if it shows any blackening of the indicator paper strip inside the bottle, bag or test tube.

A negative control should also be prepared for each new source of distilled water used and for each batch of the culture medium prepared. The negative control is prepared in order to determine that the distilled water and lab-prepared reagent used are adequate for sampling purposes.

The following Table 29 presents a rough interpretation results for the H_2S test. However, throughout this study, the H_2S test results were not assigned numerical value such as >10/100 mL, >50/100 mL or >100/100 mL (such as the table presented here suggests), but rather were considered as qualitative, P/A results.

Table 29. Interpretation results for the H_2S test. (Adapted from (IDRC, 1998).

Volume of sample with a positive result	Amount of bacteria per 100 mL	Observations

10 mL	10 or more indicator bacteria	Probably more than 100 bacteria/100 mL if the blackening takes place very fast and very intensively (less than 18 hours)
20 mL	5 or more indicator bacteria	Probably more than 50 bacteria/100 mL if the blackening takes place very fast and very intensively (less than 24 hours)
100 mL	1 or more indicator bacteria	Probably more than 10 bacteria/100 mL if the blackening takes place very fast and very intensively (less than 24 hours)

Disposing of used H_2S tests

Once H_2S samples have been interpreted, the samples can be disposed of by adding a few drops of household bleach (typically about 6% chlorine concentration). The samples must be allowed to sit for 30 minutes. The sample can be disposed down a drain, a latrine, or a dug hole, and the H_2S paper strip reagent can be disposed of as waste.

Appendix D. Easygel® Procedure

*As prepared by Stephanie Trottier.

The Coliscan® Plus Easygel® is an enumerative test with a selective substrate for *E.coli* and other coliforms. The following instructions were taken from (Micrology Laboratories, 2008).

Material

- Easygel® bottle containing Coliscan® clear medium
- Pre-treated petri dish
-

Instructions

1. Collect your water sample using a sterile container and transport back to the test site; or take a measured water sample from the source and place directly into the Easygel® bottle.
2. Remove the cap of an Easygel® bottle and, using a sterile pipette, transfer 0.5 mL to 5-mL of the sample into the Easygel® bottle without touching the sides of the bottle. Swirl gently for 1-2 minutes to distribute the sample.
3. Lift the lid of a pre-treated petri dish and pour the Coliscan®/sample mixture into the dish bottom, making sure that the entire bottom dish is covered with the liquid.
4. While the mixture is still liquid, the dishes can be placed right-side-up directly into a level incubator, or be in a warm, level area. The mixture will gel in approximately 45 minutes.
5. Incubate the samples at 35°C for 24 hours, or at room temperature for 48 hours.

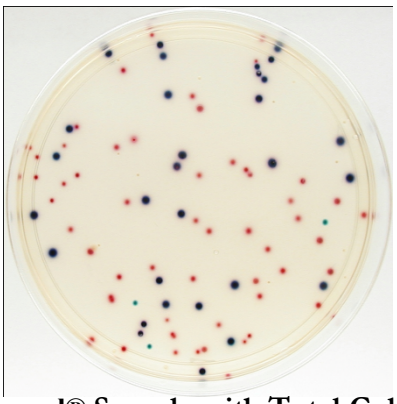
Preservation and incubation of samples

When the samples are collected directly into bottles or sterile sampling bags, these samples must be processed and incubated as soon as possible.

The Easygel® sample can be incubated in a conventional, levelelectric incubator for 24 hours, or at room temperature for 48 hours.

Interpretation of results

Count the number of red and blue colonies, disregarding any light-blue, blue-green or white colonies.*E.coli* are blue colonies and total coliform are the sum of red plus blue colonies. Below is an example of an Easygel® sample containing both total coliform and *E.coli* (red and blue colonies) The colony count will be recorded as colony forming units (CFU) per 0.5 mL to 5 mL sample, depending on the water sample volume used.



Easygel® Sample with Total Coliform and E.coli Colonies.

Disposing of used Easygel® tests

Easygel® 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 incubation.

Once samples have been interpreted and are no longer needed, the Easygel® ® sample can be disposed of in any of the following manners:

- Place dishes and Coliscan® bottles in a pressure cooker and cook at 15 lbs. for 15 minutes. Place sample in the normal trash.
- Place dishes and Coliscan® bottles in an oven-proof bag, seal it, and heat in an oven at 300°F for 45 minutes. Place sample in the normal trash.
- Place dishes and Coliscan® bottles in a large pan, cover with water and boil for 45 minutes. Place sample in the normal trash.
- Add a few drops (1 teaspoon) of household bleach (typically about 6% chlorine concentration) to the Easygel® sample and let sit for at least 5 minutes. Place the sample in a water-tight bag and discard in normal trash.

Appendix E. EC-KIT Procedure

*As prepared by Stephanie Trottier, 2010

The EC-Kit is a low-cost, field-based, microbiological testing kit comprised of two tests: the 10-mL P/A Colilert test, and the Petrifilm™ test. These instructions were taken from (Murcott & Chuang, EC-Kit Instructions, 2010).

EC-Kit material (provided in kit)

Petrifilm™

- ™*E.coli*/Total Coliform plates
- Colilert 10-mL pre-dispensed tubes
- 3.5-mL sterile plastic pipette
- Sterile sampling bags
- Incubator belt
- Black light and batteries
- Cooler bag and ice pack
- Cardboard and rubber bands
- EC-Kit instructions

Instructions

Set up and quality control procedures

1. Acquire the following materials, which are usually available locally: isopropyl (rubbing alcohol), paper towels, permanent black marker, garbage bag/masking tape or ceramic/plastic tile, soap, liquid bleach, and field notebook.
2. Wash hands thoroughly with soap and water.
3. Locate a clean, level surface and cover it 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 either work surface with isopropyl.
4. Run blanks and duplicates, for a at least 5% of total samples tested, using boiled, cooled water, or bottled water.
5. Record all test results in a lab notebook. Be sure to include date, each test result and observations.

Procedure for Colilert Test

1. Using the black-marked 10 mL guide test tube provided (the one tube with colored tape in the package), mark all the other test tubes in your kit with a permanent black marker at the same 10 mL level line.
2. Remove cap, without touching the inside of the cap with fingers or hand. Then fill the Colilert test tube with 10 mL of sample water to the black mark 10 mL level line by:
   - Filling the Colilert tube to the 10 mL mark by adding water directly, if using tap or other water supply delivered via a spout or on/off spigot (e.g. hand pump, public standpipe, treatment unit spout). Make sure you do not exceed the 10 mL black-marked level on the

tube. Replace cap and invert tube several times to mix.

- Collecting the water sample in a sterile plastic bag (provided with the kit) and either pouring directly from the bag into the Colilert tube, or using the sterile pipette provided in kit (graduated at 1 mL) to transfer sample water from the plastic bag to the test tube 10 times, taking 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.

3. Put Colilert tube in top pocket of incubator belt.

Procedure for Petrilfilm™ Test

1. Place the Petrifilm™ on a flat surface that has been wiped down with isopropyl alcohol.
2. Fill sterile pipette with 1mL of sample water (1 mL=top graduated line just below top of pipette bulb). Lift the top film.
3. With pipette perpendicular to Petrifilm™ 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 Petrifilm™ 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 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 Petrifilm™ plates can be stacked between one set of cardboard squares.

Preservation and incubation of samples

When the samples are collected directly into bottles or sterile sampling bags, these samples must be processed and incubated as soon as possible.

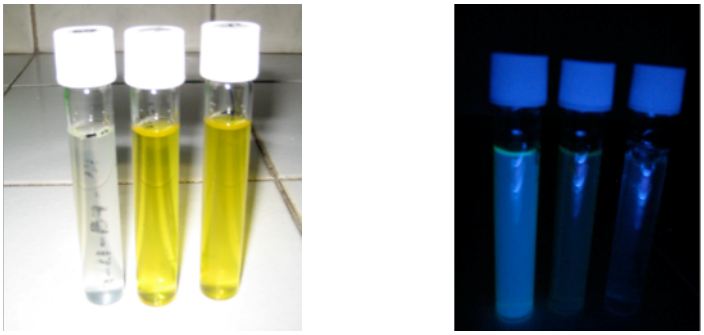
Place the Colilert tube in the top pocket of the incubator belt, and the Petrifilm™ (between two pieces of cardboard fastened with rubber bands) in the bottom pocket of the incubator belt.
Tie the incubator belt around your waist. The incubator belt must be worn continuously for 24 +/- 2 hours. This will incubate the water samples using body heat.

Interpretation of results

Interpreting Colilert results

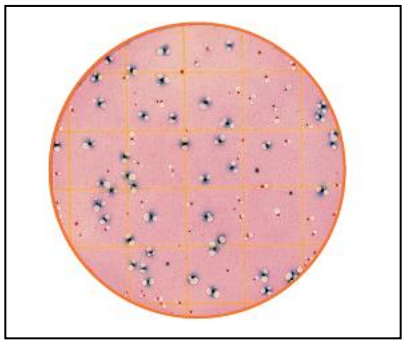
- After 24 hours, if samples are clear, no coliform bacteria are present If samples are slightly yellow or yellow, coliform bacteria are present Record as clear (absent) or yellow (present) on data sheets.
- If the samples fluoresce to form a milky-blue color under UV/black light, then *E. coli* are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

NOTE: The middle and right-hand tubes in Figure 1b show UV/black light reflecting off the Colilert tube glass. This is not fluorescence. If *E.coli* are present, a Petrifilm™ test should also be performed in order to quantify *E.coli* colonies (If sample risk is unknown, perform both tests).



Colilert tube test results after 24-hour incubation, under regular light and UV/black light.

Interpreting Petrifilm™ *Results*
Count the number of red and blue colonies with gas bubbles. *E.coli* are blue colonies with gas bubbles, and total coliform are the sum of red plus blue colonies with gas bubbles. Below is an example of a Petrifilm™ sample with total coliform and *E.coli* (red and blue colonies with gas bubbles) The colony count will be recorded as colony forming units (CFU) per 1 mL sample.



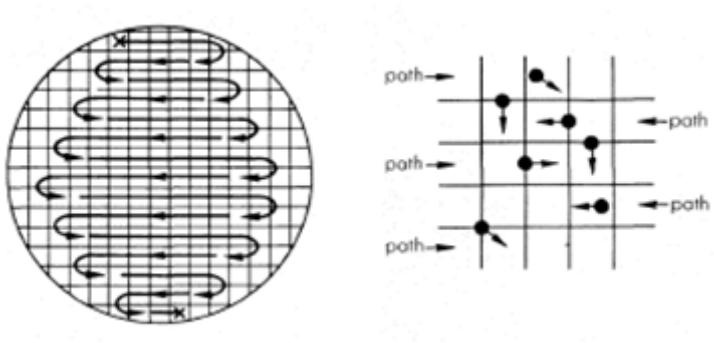
Petrifilm™ Sample Containing Total Coliform and *E.coli.* (3M, 2011)

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, fluorescence 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
- 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.



System for Counting Coliform Colonies

Disposing of used EC-Kit 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 samples have been interpreted and are no longer needed, add a few drops of household bleach (typically about 6% chlorine concentration) to the Colilert and Petrifilm™ samples (by lifting the film). The samples must be allowed to sit for 30 minutes. The Colilert can then be disposed down a drain, a latrine, or a dug hole, and the Petrifilm™ can be disposed of as waste.

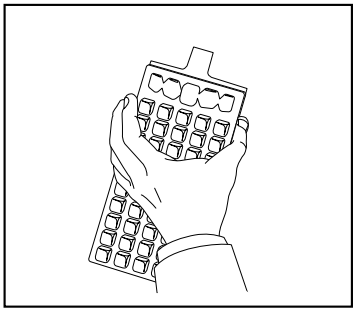
Appendix F. Quanti-Tray Procedure

*As prepared by Stephanie Trottier, 2010

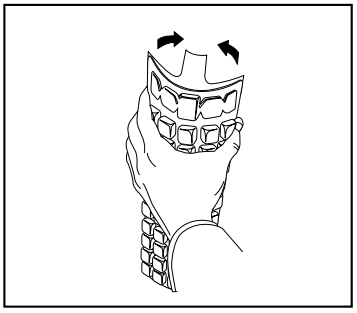
The IDEXX Quanti-Tray® is a Standard Methods that uses the enzyme-substrate method to give enumerative bacteria counts of 100 mL samples using IDEXX Defined Substrate Technology™ reagent products. The following instructions were taken from (IDEXX)

Instructions

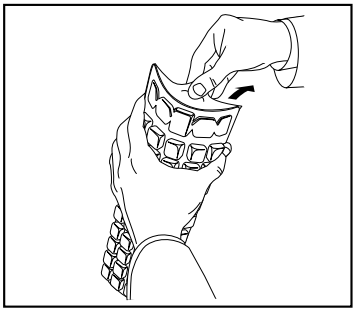
1. Add the powdered reagent to 100 mL of sample. Shake sample until powder has completely dissolved.
2. Add the reagent/sample mixture to a Quanti-Tray®, seal it in a Quanti-Tray® Sealer



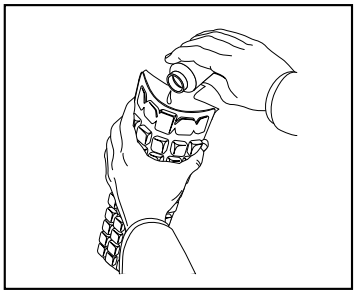
1. Using one hand to hold a Quanti-Tray® upright with the well side facing the palm



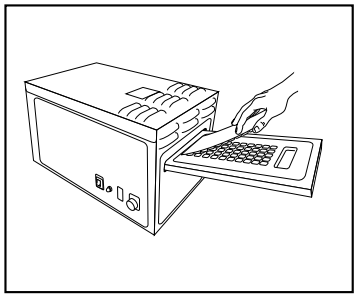
2. Squeeze the upper part of the Quanti-Tray® so that the Quanti-Tray® bends towards the palm.



3. Open the Quanti-Tray® by pulling the foil tab away from the well side. 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. Allow foam to settle.



5. Place the sample-filled Quanti-Tray® onto the rubber tray carrier of the Quanti-Tray® sealer with the well side (plastic) of the Quanti-Tray® facing down to fit the carrier.

Preservation and incubation of samples

When the samples are collected directly into bottles or sterile sampling bags, these samples must be processed and incubated as soon as possible. The Quanti-Trays® should be incubated for 24 and 18 hours for Quanti-Tray® and Quanti-Tray®/2000, respectively.

Intepretation of results

Count the number of positive (yellow) wells for total coliform and the number of positive (fluorescing under UV/black light) wells for *E.coli*. Use the appropriate Quanti-Tray® MPN table to determine the Most Probable Number (MPN) for total coliform and *E.coli* per 100 mL.

Disposing of used Quanti-Tray® tests

Quanti-Tray® 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 18 or 24 hours (for Quanti-Tray® and Quanti-Tray®/2000, respectively) of incubation.

Once samples have been interpreted and are no longer needed, the Quanti-Tray® tests can be disposed of by incinerating them, or by sterilizing them in an autoclave, before disposing of them in the normal trash.

Appendix G. A Proposal for Further Verification of H_2S Producing Bacteria as Indicator Organisms

**Prepared by (Samantha O'Keefe, 2011)

Summary

This study will evaluate members of the guild of sulfate reducing bacteria (SRBs)[5] as alternative indicator organisms for human fecal contamination in water sources. An indicator organism is a non-pathogenic bacteria that can be used as a proxy for harmful bacteria in, for example, aquatic ecosystems. The ease and relatively low cost of indicator organism testing allows for more frequent water quality monitoring, allowing bacterial contamination to be detected with greater ease and at a higher accuracy.

Currently, SRBs are being explored as possible replacements for coliforms and *E. coli*, the most commonly used indicator organisms. Though coliforms are thought to be the dominant group of bacteria that exist in the gut and feces of warm-blooded animals, a more specific test for bacteria present in the human gut and excrement would be more accurate. Additionally, coliforms and E. coli have been found to persist naturally in tropical waters regardless of the presence of warm-blooded animal species.

To address these issues we will first investigate which SRBs are present in the human gut and thus are relevant for detection in aquatic environments with which humans interact by, for example, drinking or swimming. Utilizing DNA profiles provided by the Human Microbiome Project as well as GenBank we will determine if there are SRBs present and if so, what specific species exist in the human gut.

Next, a number of pristine water sources in which SRBs are likely to be found due to environmental conditions will be selected. We will extract and amplify DSR and 16S rRNA sections of the genomic community using PCR and compare the oligonucleotides to those available in the GenBank to determine the types of SRBs present.

From the information gathered in these steps, an appropriate indicator specie(s) of SRB will be identified and a PCR diagnostic developed. An environmental interview will be conducted using the diagnostic test at various environmental sites and the performance of the test quantified compared with standard methods.

The outcome of this research could provide the global community a new, more reliable indicator organism to be used in water testing and increase the global accuracy of microbial water quality data.

Specific Aims

There are currently seven criteria recommended by the World Health Organization (WHO) for determining an ideal indicator organism. Each of the following aims addresses whether SRBs meet two of the seven criteria, namely are the indicators present in contaminated samples and absent in uncontaminated samples, and are they readily detectable by simple, inexpensive methods.

Aim 1: To identify one or more specific species of SRBs as candidate alternative indicator organisms, which exist in high concentration in the human gut, but do not exist or persist naturally in aquatic environments.

[5] In this proposal SRBs refer to the same species of organisms classified as H_2S producing bacteria in the main document.

1. A. Identify specific species of SRBs that exist in high concentration in the human gut.

1. A. a. Making use of the data collected by the Human Microbiome Project and the culture independent sequences in GenBank, what are the specific species that existent in the human gut.

1. B. Determine which species of SRBs are present in a variety of pristine aquatic ecosystems.

1.B.a Using samples from selected pristine environments, presence of any SRBs, using a FISH probe for the nucleic acid sequence that encodes dissimilatory sulfite reductase, a key gene involved in sulfate respiration.

1. C. SRBs identified in aquatic environments will be compared with the SRBs found in the human gut. The species that are present in the gut and not in aquatic environments are candidates for alternate indicator organisms.

Aim 2: To create and test a PCR diagnostic test for human fecal indicators, and complete preliminarily field tests.

2.A. A diagnostic test will be created by developing a probe with the ability to identify highly conserved sections of the specific SRB species selected as our indicator.

2.B A field interview will be completed in order to test the fecal presence SRB diagnostic test in natural aquatic environments.

3. Research Strategy and Methods

A. Significance

Microbial contamination, caused by the introduction of animal feces to a water source, is the most common threat to water quality in developing countries. Fecal indicators, defined by the World Health Organization (WHO) as "a group of organisms that indicates the presence of fecal contamination," are used as proxies to detect fecal contamination. The ease and relatively low cost of indicator organism testing allows for more frequent testing, which can allow bacterial contamination to be detected with greater ease and at a higher frequency.

To date, no organism has been identified that perfectly fulfills the criteria set out by the WHO for the "ideal indicator." Currently, total coliforms and E. coli are the most commonly used indicator organisms. Both have been detected in high densities in pristine sources in tropical waters and thus in some environments do not meet the requirement to persist in water in a manner similar to fecal pathogens. In addition, current tests have an inability to distinguish between bacteria originating from the human gut versus that of other mammal species.

In 1982, Manja, Maurya and Rao observed that the presence of coliforms in drinking water was associated with hydrogen sulfide-producing organisms (Manja 1982). Since that initial observation, extensive research has been conducted regarding the correlation between the presence of coliforms and sulfate reducing bacteria Most research has focused on comparison between tests and detection methods, as well as the accuracy and feasibility of proposed tests for H_2S -producing bacteria (Review: Trottier 2010). However, no research has yet studied which organisms the test detects, and there is concern that the test is susceptible to false positives if it detects H_2S -producing bacteria that occur naturally in the environment.

If the specific SRBs present in solely in the human gut are able to be identified, isolated and quantified, a test could be developed that would no longer produce the false positives associated with today's H_2S producing bacterial test. In addition, populations that rely on tropical waters

would no longer be excluded from indicator organism testing methods that are almost always available at a lower cost than testing directly for pathogens themselves.

B. Approach

Aim 1: To identify specific species of SRBs as candidate alternative indicator organisms, which exist in high concentration in the human gut, but do not exist or persist naturally in aquatic environments.

Part 1-Human Gut, SRB Data Collection

The Human Microbiome Project (HMP) is an NIH funded initiative whose goal is to characterize the human microbial community and use this information to better inform health and disease treatment and related fields. HMP is sampling and analyzing the metagenomes of five sites of the body; nasal passages, oral cavities, skin, urogenital and most importantly for this study, the gastrointestinal tract.

Due to the nature of the interview, there are limited confidence levels associated with taxonomic assignments and therefore HMP only releases taxonomies of those species that are highly certain. These highly certain species presented are known as reference cases, and were observed, identified and sequenced with culture-based methods. Because many microbes are known to be un-culturable, the set of reference genomes does not represent a complete interview of the species present in the human gut.

A deeper and more comprehensive metagenomic interview of microbes of the human gut, which did not rely on culture-based methods, was performed by Qin et al. (2010). The data set produced from this study, which is publicly available, could contain data that would be useful to our interview, should our initial use of the species published by the HMP prove to be insufficient to identify a candidate indicator organism. Analysis of this data is addressed at the end of this section (Aim 1).

Part 2-Selection and Sampling of Natural Aquatic Environments

A. In order decide the natural environments to be studied, the following guideline criteria were used to evaluate if an ecosystem would produce a valid result.

A.1 Expected Presence of SRBs

SRBs will not be abundant in all aquatic systems and therefore environments must be selected based on a strong likelihood that sulfate can and will act as the terminal electron acceptor in metabolic processes. This occurs in locations with limited availability of what may be considered the standard sources of carbon, electrons and electron acceptors; for example, anoxic environments.

A.2 Extremely low probability of human fecal contamination.

The method by which species will be eliminated as viable indicators for contamination is if they are present in natural waters where there is no fecal contamination. Therefore, the sites should be located at a distance from any wastewater treatment facilities, and preferably at an elevated altitude to minimize the potential for fecal contamination from runoff.

A.3 Variation between selected environments.

As the focus of the study is to find SRBs appropriate as worldwide fecal indicators, environmental SRB detection should be performed in a variety of aquatic environments. Varying factors including salinity, temperature, turbidity of the water etc.

A.4 Sampling feasibility and access to selected environments.

While a number of environments fit the previous three criteria, deep-sea hydrothermal vents as well as several pristine environments in the Antarctic and Artic are unfeasible for our group to sample.

B. Based on the above criteria, the group plans on sampling the following environments for SRB content:

B.1.Saturated subsurface sediment at Farm River Estuary, New Haven County, CT

Sediment cores to a depth of at least 60 cm will be taken from a small number of pristine sites within the estuary, with the goal of isolating bacteria from the sulfate reduction zone, at depth where sulfate reduction becomes more favorable relative to oxygen, nitrate, and iron. The close proximity of Farm River Estuary to the group's facilities will allow for relatively inexpensive sampling.

B. 2.Anoxic chemocline waters from Waldsea Lake, Saskatchewan, Canada
High levels of sulfate have been documented in some of the salt lakes of the northern Great Plains in western Canada (Last and Vance, 1996). Water samples will be taken using a gas-tight sampler and transported to the laboratory for detection of SRB's.

Part 3-Sequencing and Identification of environmental species

Following sample collection from the above pristine environments, the samples will be evaluated for the presence of sulfate reducing bacteria using a fluorescent in situ hybridization (FISH) probe for the genes that encode dissimilatory sulfite reductase (DSR), a key enzyme in sulfate respiration. DSR analysis is useful because DSC catalyzes reduction of sulfite to sulfide, and is therefore highly conserved and required by all SRB, even those not phylogenetically related.(Minz et al, 1999). Traditional 16S rRNA analysis is less useful in this study, because it does not provide a direct link to an organism's physiology.

Instead, a single primer set, identified by Wagner et al. (1998), will be used to amplify the conserved region of genes encoding DSR. This primer set, which we will request from Wagner et al., amplifies a 1.9-kb DNA fragment that encodes most of the alpha and beta subunits of the DSR in all recognized lineages of SRB (Wagner et al, 1998). This analysis is useful because "DSR catalyzes the six-electron reduction of sulfite to sulfide and hence is required by all SRB. Development of a general PCR primer set was possible because of the remarkable conservation of the DSR sequence." (Minz et al, 1999). The sequences of PCR products of this amplification will be compared to data in GenBank, to determine which species are present in each environment.

Sampling procedures, nucleic acid extractions, PCR and sequencing will be carried out as in methods described by Minz et al. (1999).

Part 4 - Identification of candidate indicator organism by comparison of Parts 1&2

Next, we will compare the SRBs identified in aquatic environments with the SRBs found in the human gut, based on their sequenced DSR regions. We expect to encounter at least one, but

possibly a short list of SRBs that exist in the human gut, but are not found in any of the sampled pristine aquatic environments. These species are candidates for alternate indicator organisms, and there use as such will be preliminarily evaluated by Aim 2.

It is possible that all species of SRBs identified in the human gut that are included in the reference strains given by the HMP are also present in aquatic environments. Should this be the case, we will turn to the previously mentioned data set published by Qin et al. (2010). This dataset contains the metagenomic DNA sequencing of fecal samples from 124 adults, for a total of 576.7 Gb of data. If necessary, we will search this dataset to find DSR genes, based on the probe sequences specified by Wagner (1998). We will then compare these DSR sequences (which represent species of SRB that exist in the human gut) to the sequences of the DSR amplification products from the aquatic environment species. Should this analysis result in DSR sequences which, once again, are present in the human gut but not in the environments sampled, candidate indicator species will have been identified. However, their only identifying factor will be their DSR sequence, which may or may not provide enough information to complete Aim 2.

If this additional analysis still does not identify at least one candidate indicator organism, it indicates that SRBs may not be ideal indicators; that is, there may not exist a species of SRB that exists exclusively in the human gut. However, further research regarding geographic and environmental distribution could be useful for developing location-specific diagnostic tests.

Aim 2-To create and test a PCR diagnostic test for human fecal indicators, and preliminarily test it in the field.

Part 1-Development of a diagnostic.

From the comparison of the relative presence of SRBs in the human gut and a variety of aquatic environments, a single or multiple species will be identified as appropriate indicator for human fecal contamination. In the case where a species is not able to be identified as meeting all of the above criteria, the next best species will be chosen as the potential indicator, where next best is defined as the species which appeared least frequently in the environment, and only appeared in environments that are not generally inhabited by humans.

From the candidate indicator species, a sequence of conserved DNA that is specific to the species will be selected. This sequence will first be searched for within the 16S rRNA and DSR genes, given that they are highly conserved regions with relevance to other aspects of this project. A pair of PCR primers will be constructed to amplify this region, and this pair of primers will function as a PCR probe for the presence of the candidate indicator organism.

Part 2-Environmental field tests

Having designed an appropriate probe, a series of field tests will be performed to preliminarily indicate its success. Samples from the aforementioned pristine environments will be included, as well as sites that are likely or known to have high levels of fecal contamination, such as aquatic environments downstream of wastewater discharge, lakes in densely populated urban areas. A sample of untreated wastewater and a sample of tap water will also be included in the sample set, as positive and negative tests, respectively.

DNA from these sites will be extracted using the methods described by Minz et al. (1999). A standard DNA extraction and PCR-based diagnostic will be performed, using the newly designed primer pair. Raw samples from the sites will also undergo the commonly used, EPA certified tests for total coliforms and E coli.

A successful probe will indicate the presence of the candidate indicator organism in the sample of untreated wastewater, and the absence of the candidate indicator organism in samples from the pristine environments. Positive test results, in which the indicator organism is detected, are expected to correlate with, if not match, those of the total coliform and *E. coli* tests. Success of the probe indicates a successful identification of a possible candidate indicator organism, and the possibility of a new, functional worldwide fecal contamination detection test.

If the environmental interview results prove positive, subsequent laboratory testing will be proposed for further verification of the use of the identified species as a fecal indicator organism, according to the requirements set out by the WHO.

Overall

To date, research in the field of indicator organisms has focused primarily on the comparison of newly developed tests to industry standards. While, in the end similar verification will take place, the proposed study will take a more aca (Last, 1997) (Minz, 1999) (Qin, 2010)demically rigorous approach in developing the diagnostic test. This is to be done by identifying SRBs that are unique to the human gut and do not exist in environments in which there is no fecal contamination. Once these organisms are identified, the diagnostic will be developed.

Appendix H. Household Survey Questions

Water Source/Storage

1. What is the primary source of your drinking water?
2. Do you share this source with other members of your community?
3. What is the primary container used for storage of your drinking water?
4. Do you clean the storage container?
    a. If yes, how often?
    b. If yes, with what do you clean the container?
5. Do you pay for access to this water source?
    a. If yes, how much?
6. How reliable is your drinking water source?
7. Do you use the same drinking water source year round?
    a. If no, what is the other source of drinking water?

Water Treatment/Use

1. Do you filter your water in any way?
    a. What type of filter do you use?
    b. How long have you filtered the water?
    c. If not, have you used a filter in the past?
    d. Where did you get the filter?
    e. Why did you stop using the filter?
2. Do you treat the water in any other way?
    a. If yes, what do you do to it?
    b. Where do you purchase the materials?
    c. How much do you pay for the materials?
    d. If no, have you treated the water in the past?
    e. What did you use?
    f. Why did you stop?
3. What is the main use of the water you collect?

Sanitation/Health

1. Are there latrines in the community?
    a. If yes, do you use them?
    b. If yes, do you prefer them to the bush?
    c. If yes, why?
2. Are there any major illnesses in the village?
    a. If yes, what are they?