PERFORMANCE EVALUATION OF THE COMPARTMENT BAG TEST
FOR E. COLI IN DRINKING WATER

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ABSTRACT

Alice Wang: Performance Evaluation of the Compartment Bag Test for *E. coli* in Drinking Water
(Under the direction of Mark D. Sobsey)

Nearly 748 million people worldwide lack access to improved drinking water sources, putting them at risk for waterborne illnesses. Fecal contamination of drinking water is one of the largest contributors to the 1.8 million deaths per year from diarrheal disease. Because many countries lack active monitoring of drinking water quality, it is often unknown if consumed waters are safe. Current microbial monitoring methods typically require the use of specialized equipment, electricity, and trained personnel. However, in low-resource settings, these capacities are often unavailable. Therefore, there is a need for a low-cost, portable, and simple method for determining the microbial quality of drinking water in low-resource settings.

The drawbacks of current water tests based on fecal indicator bacteria may be overcome using the Compartment Bag Test (CBT), a novel microbial water quality test innovated at the University of North Carolina at Chapel Hill. The CBT uses a simple design of a clear, chambered plastic bag with various compartment volumes totaling 100 mL to determine a Most Probable Number (MPN) estimate of *Escherichia coli* bacteria concentration using a chromogenic liquid medium. This semi-quantitative method could provide actionable results to identify microbially unsafe water and decrease microbial water quality health risks, if its performance is further documented against standard tests under a variety of use conditions.
The goal of this research is to evaluate and document the performance of the CBT. A laboratory evaluation was conducted to explore the use of CBT to detect *E. coli* compared to a standard test using the Colilert medium in Quanti-Trays at various incubation temperatures. The CBT was also evaluated in field settings by incorporating the CBT in Demographic Health Surveys in Peru and Liberia. Household surveys were conducted in Tanzania to evaluate the CBT as a health behavior and education tool. Overall these studies demonstrate that 1) the CBT detects and quantifies *E. coli* comparable to standard methods, 2) incubation temperature between 27°C to 44°C provide comparable *E. coli* MPN results, 3) the CBT can be utilized in low resource settings and incorporated within national health surveys, and 4) the use of the CBT as a health behavior and education tool can influence perception and knowledge of microbial water quality of household users.
I am truly grateful and would like to acknowledge the support of my doctoral dissertation committee. Thank you to Dr. Orlando Coronell, Dr. Jennifer Horney, Dr. Michael Emch, Dr. Jill Stewart and Dr. Mark Sobsey. I am humbled by the opportunity to work with such wonderful professors and great minds in science! Thanks to my doctoral dissertation committee, throughout my dissertation research I was able to be detail oriented and yet see how the experiments I was conducting can fit into a larger public health picture. I am appreciative of their encouragement, mentorship, and advice throughout my time as a doctoral student.

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I could not have accomplished this research without the incredible assistance of my collaborators. In Peru, I want to acknowledge Meleny Katherine Puris Condor; Erica Cerron Garcia; Maria Yolanda Ramirez; Jorge Reyes Morano and Prudencia Javier from the Instituto Nacional de Estadística e Informática; Eddy Rojas from La direccion ejecutiva de Salud Ambiental laboratorio; Margarita Molina from the Instituto de Investigacion Nutricional; Gladys Nahir Chuquipiondo Laulate and Dina Chong Vasquez from the Asociacion Civil Selva Amazonica; and Dr. Shea Rutstein From ICF International. In Liberia, I want to acknowledge Henry Bundor; Alex Leinkpor; Patricia Morrison; Grace Gwesa, Aletha Nana, and Edwin Fallah from the Water Quality Control Laboratory of the Ministry of Health and Social Welfare of Liberia; and Dr. Joanna Lowell from ICF International. In Tanzania, I want to acknowledge Robert Masunya, Yusuph Bunzali, Gogadi Mgwatu, and Maryam Mshana from the Mwanza Urban Water and Sewerage Authority; Dr. John Feighery of mWater; and Lars Stordal from UN-Habitat.

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needed to talk through ideas, read and edited through pages of my papers and fellowship applications, and supported me even while I was conducting research on another continent.

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<table>
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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>CBT</td>
<td>Compartment Bag Test</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>DALYs</td>
<td>Disability-adjusted life years</td>
</tr>
<tr>
<td>DHS</td>
<td>Demographic and Health Survey</td>
</tr>
<tr>
<td>EC-MUG</td>
<td>4-methylumbelliferyl-β-D-glucuronide</td>
</tr>
<tr>
<td>FC</td>
<td>Fecal Coliform</td>
</tr>
<tr>
<td>GEMS</td>
<td>Global Enteric Multicenter Study</td>
</tr>
<tr>
<td>GBD</td>
<td>Global burden of disease</td>
</tr>
<tr>
<td>H$_2$S</td>
<td>Hydrogen Sulfide</td>
</tr>
<tr>
<td>INEI</td>
<td>Instituto Nacional de Estadística e Informática</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
</tr>
<tr>
<td>JMP</td>
<td>Joint Monitoring Programme</td>
</tr>
<tr>
<td>LTB</td>
<td>Lauryl Tryptose Broth</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>Matrix Assisted Laser Desorption/Ionization Time of Flight</td>
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<tr>
<td>MDG</td>
<td>Millennium Development Goals</td>
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<td>MICS</td>
<td>Multiple Indicator Cluster Survey</td>
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<tr>
<td>MPN</td>
<td>Most Probable Number</td>
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<tr>
<td>MS</td>
<td>Mass Spectrometry</td>
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<tr>
<td>MWAUSA</td>
<td>Mwanza Urban Water and Sewerage Authority</td>
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<tr>
<td>NGO</td>
<td>Non-governmental organization</td>
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PBS  Phosphate Buffered Saline
PCR  Polymerase Chain Reaction
POU  Point of use
qPCR  Quantitative Polymerase Chain Reaction
RT-qPCR  Real-time Quantitative Polymerase Chain Reaction
spp  Species
TC  Total Coliforms
TSA  Tryptic Soy Agar
UN-Habitat  United Nations Human Settlements Programme
UNICEF  United Nations International Children’s Fund
USD  United States Dollar
USAID  United States Agency for International Development
WASH  Water, sanitation and hygiene
WHO  World Health Organization
X-Gluc  5-bromo-4-chloro-3-indolyl- β-D-glucuronic acid
CHAPTER 1: OVERVIEW AND OBJECTIVES

1.1 Introduction and Background

*Global drinking water issues and the Millennium Development Goal target*

It is estimated 748 million people worldwide lack access to safe drinking water sources, putting them at risk for water-borne illnesses, especially diarrheal disease (WHO/UNICEF, 2014). Fecally contaminated drinking water is one of the largest contributors to the 1.8 million deaths per year from diarrheal disease. The vast majority of these deaths occur in children under five years of age because they are especially susceptible to the effects of diarrheal disease. In developing countries, the lack of safe, managed water sources, and water infrastructure results in the use and consumption of water from unprotected, compromised, and potentially polluted sources (Brown et al., 2008; Lee et al., 2005; Wright et al., 2004). Reviews of public health interventions to prevent diarrheal disease have documented median reductions in diarrhea from water quality interventions ranging from about 15 to 40%, (Clasen et al., 2007; Esrey et al., 1991; Fewtrell et al., 2005).

In 2010, the United Nations Human Rights Council, in its Resolution A/HRC/RES/15/9, affirmed that the “right to water and sanitation is derived from the right to an adequate standard of living and inextricably related to the right to the highest attainable standard of physical and mental health, and the right to life and human dignity” (WHO/UNICEF, 2012). Because access to safe drinking water is acknowledged as a human right, the importance of monitoring equity in that access is increasingly recognized. Therefore, monitoring water quality, specifically
microbial water quality, is imperative in distinguishing safe and unsafe water and then preventing unsafe water with directed interventions, identifying and responding to outbreaks of waterborne illness, tracking the sources of contamination to then intervene and minimize exposure, assessing the effectiveness of water disinfection and distribution programs, as well as ensuring safe water a human right.

One of the aims of the Millennium Development Goals (MDG), as set forth by the United Nations in 2000, Target 7c, is to halve by 2015 the number of people without access to safe drinking water. For the MDG target, the definition of an improved or unimproved water source was based on the water source and/or the type of technology employed at a water point, see Figure 1.1, rather than actual sampling and analysis of the drinking water. This approach to classifying water access was due to the cost and complications that microbiological water testing entails (WHO/UNICEF, 2012). Global access to improved water sources has increased since 1990, due in large part to the successful mobilization of resources organized in support of the MDG, and Target 7c is considered to be “on track” and was met in 2010, five years ahead of schedule (WHO/UNICEF, 2012).

Figure 1.1 Definition of improved/unimproved drinking water sources by the Joint Monitoring Programme (WHO/UNICEF, 2012)
However, evidence from the UNICEF Progress on Drinking Water and Sanitation report of 2010 noted that across multiple countries, one in two protected dug wells was microbially contaminated, and one in three protected springs and boreholes were microbially contaminated (WHO/UNICEF, 2010). Because of these findings, the “Improved” source indicator for drinking water has received criticism for not adequately reflecting safety and acknowledgement from the JMP (Godfrey et al., 2011; Bain et al., 2012b; WHO, 2011). Other recent evidence, such as the rapid assessment of drinking-water quality projects (RADWQ) and conclusions from global modeling projections based on RADWQ data has also called into question the microbiological safety of many water sources that are considered improved under the definitions used by the Joint Monitoring Programme (JMP) (Bain et al., 2012b; Onda et al., 2012). Also, regional disparities in safe water access still exist. In order to decrease these disparities, it is necessary to identify where drinking water is microbially unsafe before work can be done to improve it (Godfrey et al., 2011). The consumption of contaminated drinking water sources is most prevalent in Africa and Southeast Asia (Bain et al., 2014). There is still more diarrheal disease due to drinking water in remote versus non-remote areas (Eisenberg et al., 2006; Bain et al., 2014). In response to these concerns, the JMP has proposed a water quality target for the post-2015 Sustainable Development Goals (SDGs). The SDGs include a measurement of the fecal indicator bacterium, *Escherichia coli*, in water for monitoring basic and intermediate water service levels, with the intermediate level defined by the absence of *E. coli* at levels above 10CFU/100 mL (Bain et al., 2014).
Limitations of water quality testing in low-resource settings

Currently approved methods for the enumeration of *E. coli* in drinking water samples require the use of specialized equipment, including an electrically powered incubator, and entail complicated procedures that must be performed by trained personnel (Bain et al., 2012a). Many areas of the world that lack access to improved drinking water sources are located in remote rural regions where little or nothing is known about the microbial quality of drinking water sources used by communities and households. In such low-resource settings, which may also be very isolated, accessible methods for determining the microbial quality of drinking water sources are lacking. Furthermore, standard methods used to monitor microbial water quality for regulatory compliance in even developed countries may be extremely difficult to use in these types of settings (Bain et al., 2012a). For example, the US EPA procedures for microbial analysis of water samples state that the sample must be kept between 1-4°C during transit and should be analyzed as soon as possible after collection and not more than 30 hours post-collection (US EPA, 1982). It is difficult to achieve these sample-handling conditions in low-resource settings and the quality of analysis of a sample that does not follow these guidelines will potentially be compromised. Therefore, there is a need for a low-cost, portable, simple method that does not require specialized and highly skilled analysts, additional equipment and materials, such as an incubator, and can be performed on-site, to determine the microbial quality of drinking water in low-resource settings (Bain et al., 2012a; Onda et al., 2012).

The Compartment Bag Test as a novel Method for microbial water quality analysis

A novel Compartment Bag Test method (CBT) for quantifying *E. coli* in drinking water samples has the potential to overcome the barriers to microbial water quality testing in low
resource settings (Bain et al., 2012a; McMahan et al., 2009; 2011). The CBT is portable, simple to perform with few steps, can be visually scored, requires no cold chain or supporting equipment and specialized materials, and can be performed on-site. The CBT is a polyethylene bag (Whirl-Pack, Nasco) that was modified to provide separate internal chambers of 56, 30, 10, 3, and 1 mL sample volumes, totaling 100 mL. A Hi-\textit{E. coli} test bud of \textit{E. coli} bacteriological medium (HiMedia Labs, Mumbai, India) containing a chromogenic glucuronide substrate, 5-bromo-4-chloro-3-indolyl-\textbeta-D-glucuronic acid (X-gluc), is added to a water sample and the amended water is swirled to fully dissolve the medium. Once the medium reagent is dissolved, the sample is transferred to a sterile CBT. The sample is then distributed among the 5 compartments by tilting the bag from side to side and manual adjustment (squeezing) of the compartment volumes. An external 2-piece spring plastic clip is placed across the bag above the liquid levels in the compartments but below the tops of the compartments in order to isolate the compartments from each other. The sealed bag is then incubated at 27-44.5\textdegree C for 18-24 hours, or longer at the lower temperatures of 27-30 \textdegree C, and the compartments that show bacterial growth by the presence of any trace of blue or blue-green color are considered to be positive for \textit{E. coli} growth. The combination of positive compartments and their volumes provide the basis for an MPN estimate of the \textit{E. coli} concentration per 100 mL of water that is looked up in a table (See Appendix 1).

1.2 Objectives

The purpose of this research is to evaluate the CBT for detecting and quantifying \textit{E. coli} in drinking water samples both in the laboratory and in field settings. Much of the previous research on the CBT has been conducted with a medium containing lauryl tryptose broth (LTB)
with the addition of X-gluc. A new and improved medium for the detection of *E. coli* has been produced and will be further tested in the laboratory. Also, while previous laboratory based experiments on the CBT have been conducted, there is the potential for differences between laboratory effectiveness and efficacy in the field. This proposed research is largely aimed at assessing the efficacy and applicability of this new method for use in low-resource settings in the field compared to other currently available and accepted methods. The CBT will be evaluated both in the laboratory and field for its performance in *E. coli* detection in drinking water and its sources. The specific objectives are outlined below.

1. **Laboratory validation of CBT detection of *E. coli* in water samples**
   a) Compare CBT media with Colilert in detection and quantification of *E. coli*
   b) Evaluate CBT detection results at various incubation temperatures
   c) Identify presumptive positive and negative *E. coli* isolates from the CBT

2. **Field application of CBT within a Demographic Health Survey in Peru**
   a) Compare the CBT when used in the field by surveyors and in the laboratory by trained technicians to membrane filtration done in in the laboratory by trained technicians
   b) Evaluate the robustness, effectiveness and applicability of the CBT in low resource settings and when incorporated within a national health survey at pilot scale

3. **Field application of CBT within a Demographic Health Survey in Liberia**
   a) Compare the CBT when used in the field by surveyors and in the laboratory by trained technicians to membrane filtration done in the laboratory by trained technicians
b) Evaluate the robustness, effectiveness and applicability of the CBT in low resource settings and when incorporated within a national health survey at pilot scale

4. Evaluation of the CBT as a health behavior and education tool in Tanzania

a) Observe the ease and usability of the CBT by household users, and its impact on user perceptions of and attitudes about water quality

b) Evaluate the CBT as a health behavior and education tool for influencing knowledge, attitudes, and perception on water and sanitation issues
CHAPTER 2: LITERATURE REVIEW

2.1 Burden of Disease Due to Inadequate Water, Sanitation, and Hygiene

A massive health burden arises as a consequence of lack of access to safe water, sanitation, and hygiene (WASH) (Feachem, 1983; Bartram and Cairncross, 2010; Bain et al., 2014). Currently about 748 million people rely on unimproved sources of water supplies (WHO/UNICEF, 2014). Approximately 2.5 billion people still lack access to basic sanitation (WHO/UNICEF, 2014). The majority of people who use a contaminated water source live in Southeast Asia (34%) or Africa (26%), and rural areas are often more contaminated than urban areas (Bain et al., 2014). Even for populations with access to water sources and sanitation, inadequate WASH poses health risks. For example, the lower quantity of water use (Cairncross and Feachem 1993; Royal Scientific Society, 2013) and increasing distance to a water source (Tonglet et al., 1992; Galiani et al., 2007; Pickering and Davis, 2012; Evans et al., 2013) has been associated with an increased risk of diarrhea. Inadequate hand hygiene practices have been estimated to affect 80% of the population globally (Freeman et al., 2014) and are also associated with increased risk of morbidity due to diarrheal illness. Numerous health risks, including diarrheal disease, from inadequate WASH have been previously documented (Esrey et al., 1991; Fewtrell et al., 2005; Waddington et al., 2009; Wolf et al., 2014). Accounting for not only mortality, but also morbidity as measured in disability-adjusted life years (DALYs), approximately 842,000 deaths per year are estimated due to inadequate WASH, which is estimated to amount to 1.5% of the total disease burden and 58% of diarrheal diseases globally.
Several diseases are related to WASH due to pathogen transmission via water (see Table 2.1). Disease transmission by water can be classified into four categories: waterborne, water-washed, water-based and water-related (White, Bradley, & White, 1972). Ingesting fecally contaminated water transmits waterborne pathogens. Lack of adequate quantity of water for washing and bathing transmits water-washed pathogens. Water-related pathogens are transmitted via an insect vector that breeds in water. Water-based pathogens are transmitted via a parasite vector that lives in contaminated water, some of which have an intermediate aquatic host.

Table 2.1 A Summary of the description of transmission routes, examples of types of infections, and control strategies for infectious diseases associated with water

<table>
<thead>
<tr>
<th>Transmission Route</th>
<th>Description</th>
<th>Example of infections</th>
<th>Control Strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waterborne</td>
<td>Water is a passive vehicle for infectious agent; transmission is due to consumption of contaminated water</td>
<td>Bacterial infections: <em>Salmonella</em> typhoid, enterobacteria, cholera Viral infections: hepatitis A, rotavirus Parasitic infections: amoebiasis, giardiasis, intestinal protozoa, ascariasis, hookworm</td>
<td>Improvements in microbial water quality</td>
</tr>
<tr>
<td>Water-washed</td>
<td>Insufficient quantities of water for hygiene leads to infection</td>
<td>Enteric infections: diarrheal diseases and gastroenteritis Skin infections: scabies Lice-borne infections: typhus Eye and ear infections: otitis, conjunctivitis, trachoma</td>
<td>Increase water supply, improvements in hygiene practices</td>
</tr>
<tr>
<td>Water-based</td>
<td>The infective agent’s life cycle takes place in an aquatic organism; infection is transmitted through contact with contaminated water or ingestion of infective agent</td>
<td>Infections due to crustaceans: dracunculiasis Infections due to fish: diphyllobothriasis Infections due to shellfish: flukes, shistosomiasis</td>
<td>Reduce surface water contamination or contact with contaminated water</td>
</tr>
<tr>
<td>Water-related</td>
<td>Infections spread by insects that</td>
<td>Infections due to mosquitos: malaria, yellow fever, hemorrhagic fever</td>
<td>Prevent opportunities</td>
</tr>
</tbody>
</table>
breed in water
Infections due to tsetse flies:
trypanosomiasis
Infections due to blackflies:
onchocerciasis
for breeding;
Use of
barriers like
bed nets or
pesticides

| Table adapted from Wenhold and Fraber, 2009 |

The microbial quality of drinking water has a large impact on health if access to safe water is limited or lacking (Fewtrell and Bartram, 2001). Contaminated drinking water may contain unsafe levels of microorganisms that pose a risk to human health (WHO/UNICEF, 2010). Interventions in drinking water quality to reduce diarrheal disease target primarily waterborne pathogens. Waterborne pathogens comprise a broad range of microorganisms ranging from viruses to bacteria to parasites. For example, a case-control study in Ecuador documented cases of diarrhea as a result of all three classes of pathogens: *Escherichia coli*, Rotavirus and *Giardia* (Eisenberg et al., 2006). The Global Enteric Multicenter Study (GEMS), a prospective case-control study conducted in four sites in Africa and three in Asia during the years 2007 through 2011, found that most cases of moderate-to-severe diarrhea were attributable to four pathogens: rotavirus, *Cryptosporidium*, enterotoxigenic *Escherichia coli*, and *Shigella* (Kotloff et al., 2013). Other pathogens noted important in selected GEMS sites include: *Aeromonas*, *Vibrio cholerae* O1, and *Campylobacter jejuni* (Kotloff et al., 2013).

Many diseases related to WASH are zoonotic pathogens and can also infect animals, with or without causing disease in their animal hosts (Barron, 1996; FDA, 2012; Sobsey, 2015). Significant zoonotic pathogens include gram-negative enteric bacteria, including most of the *Salmonella* species, *Campylobacter* species, and disease-causing strains of *E. coli*, as well as, enteric protozoan parasites including *Cryptosporidium parvum* and *Giardia lamblia* (intestinalis) (Garcia et al., 2010; Sobsey, 2015). Helminths, such as the pork tapeworm *Taenia solium*, can
also be zoonotic and exposure and infection can result due to ingestion of contaminated water or contaminated pork (Sobsey, 2015). These zoonotic pathogens can infect many animals including livestock such as such as poultry, cattle and sheep. Infected animals often have no symptoms of disease except the fecal shedding of pathogens. Fecal shedding of pathogens from infected animals is a concern if untreated animal fecal wastes, utilized to amend soil for agriculture or discharged through irrigation, contaminate drinking water supply, produce that may be eaten raw, or waters used for bathing or recreation (Sobsey, 2015).

Pathogens transmitted through the fecal-oral route often cause diarrheal disease (Feachem, 1983). These pathogens are typically considered enteric pathogens because they can infect the gastrointestinal tract and once shed into the environment via excreta, they are capable of being transmitted in a variety of ways including through ingestion of or contact with contaminated water and person-to-person (Feachem, 1983). Diarrheal disease is a major cause of morbidity and mortality in young children and vulnerable populations. About 1 in 10 child deaths result from diarrheal disease during the first five years of life, resulting in 800,000 global deaths annually (Kotloff et al., 2013). In less developed countries, poor nutritional status and poverty exacerbate morbidity and mortality associated with excreta related diseases, as a result children with low weight for their age have a much higher risk of mortality (Carr, 2001).

Mortality from diarrheal disease is decreasing approximately 4% per year (Liu et al., 2012). However a recent study on the global burden of the disease suggests that there has not been an accompanying decrease in morbidity (Kosek et al., 2003; Kotloff et al., 2013). The average child in the developing world experiences three or more diarrheal disease episodes per year, which accounts for more than four billion cases of diarrhea annually (WHO/UNICEF, 2014). Recent estimates suggest that diarrhea, due to the consumption of contaminated water, accounts for
more than 1.8 million deaths annually (Bain et al., 2014; WHO/UNICEF, 2014).

2.2 *Escherichia coli* as a Water Quality Indicator

Monitoring for indicators for fecal pollution better protects the public’s health than monitoring for specific pathogens. There are numerous known pathogens and potentially even more unknown pathogens. The methods for monitoring pathogens are expensive, technically demanding and time-consuming (Edberg, 2000). Because the presence of pathogens correlates well with the presence of fecal contamination, current drinking water testing relies on fecal bacteria as indicators of both fecal contamination and possible presence of pathogens (Gleeson and Gray, 1996; Leclerc et al., 2001; McFeters et al, 1974; Rompre et al., 2002). In 1904, a fecal coliform test was developed for total coliforms encompassing all members of the *Enterobacteriaceae* that could ferment lactose to produce acid and gas. (Eijkman, 1904; Edberg et al., 2000). In 1914, the United States Treasury Department proposed a standard for drinking water safety that codified the utilization of a total coliform test (Clesceri et al., 1998; Edberg et al., 2000).

The total coliform test was soon criticized because it included numerous species not of fecal origin (Frost, 1915; Fuller, 1915). In 1977, a study found 96.8% of coliforms isolated from human feces were *E. coli* with the remaining 3.2% isolates identified as *Klebsiella, Citrobacter*, and *Enterobacter* species (Dufour, 1977; Tallon et al., 2005). These genera are now referred to as thermotolerant coliforms or fecal coliforms and have been used as indicators of fecal contamination of drinking water (Horan, 2003). However, some of the genera are widely found in the environment and not associated with fecal contamination. For example, not all fecal coliforms are effective indicators of fecal pollution in drinking water due to large numbers of environmental species like *Klebsiella* species (Tallon et al., 2005; Tyagi et al., 2005)
Theobald Smith first proposed *E. coli* as indicator in 1890s as the primary drinking water indicator since *E. coli* is part of the normal intestinal flora of mammals and could be found in mammal feces at concentrations of $10^9$/gram (Edberg et al., 2000). Now the indicator organism *E. coli* is the standard indicator of fecal contamination in drinking water, endorsed by the US EPA and the World Health Organization (WHO, 2011). The two key factors of why *E. coli* was chosen as indicator are: 1) the finding that some other “fecal” indicators were often non-fecal in origin, and 2) the development of improved testing methods for *E. coli* such as defined substrate technology (DST) introduced in 1987 (Edberg et al., 2000; Tallon et al., 2005). DST contains 4-methyl-umbelliferyl-B-D-glucuronide (MUG), which can be only metabolized by the enzyme, β-glucuronidase, particular to *E. coli* and present in more than 95% of all isolates of *E. coli* (Edberg et al., 2000). In DST, the substrate acts as a main food source and the metabolism of the substrate allows growth of the target microbe at the expense of others, eliminating a confirmation step. With the simplicity of DST, the detection of *E. coli* is the most widely accepted target for measuring the microbial quality of drinking water that is at risk for fecal contamination.

*E. coli* is considered an effective and reliable indicator of fecal contamination because of the following characteristics: 1) It is applicable to all types of water and other relevant samples; 2) It is present in feces, sewage and fecally contaminated samples when pathogens are present and their numbers correlate with amount of fecal contamination and outnumber pathogens; 3) It survives/persists better than or equal to pathogens; 4) It is easily detected/quantified by simple lab tests in a relatively short time; 5) It has constant characteristics; 6) It is generally harmless to humans and other animals; and the 7) It’s numbers in water, and other media such as food, are associated with risks of enteric illness in consumers with a dose-response relationship and has been shown to be associated with diarrheal disease risk (Moe et al., 1991; National Research...
However, failures to meet some of these criteria have been reported, such as the variable \textit{E. coli} to pathogen ratios in water samples analyzed during drinking waterborne outbreaks or in tropical climates (Gleeson et al., 1997; Van Lieverloo et. al., 2007; Oh et al., 2012). High concentrations of \textit{E. coli} have been found in tropical natural water systems and pulp and paper mills with no known sources of fecal contamination (Tallon et al., 2005). Despite these findings, the extent to which \textit{E. coli} fulfills the majority of the indicator criteria is considered sufficient to be preferred over other microbial indicators (WHO, 2011).

While \textit{E. coli} is considered the definitive indicator of fecal pollution, there are other potential indicators of fecal contamination that may be better suited for different environments. For example, \textit{Enterococci} can indicate bacterial pathogen presence, particularly in salt-water environments; coliphages (bacteriophages of \textit{E. coli}) indicate the presence of enteric viruses; \textit{Clostridium perfringes} indicate presence of parasitic protozoan and enteric viruses (Riesbro et al., 2012; Tyagi et al., 2005). Also, indicator bacteria do not necessarily correlate well with presence of viruses or protozoa (Noble and Fuhrman, 2001). For example previous studies have found that \textit{E. coli} is not good indicator for \textit{Cryptosporidium parvum}, \textit{Giardia lamblia}, \textit{Yersinia enterocolitica} or enteric viruses (Tallon et al., 2005). Therefore for a more accurate estimate of waterborne pathogens, \textit{E. coli}, \textit{Enterococci}, coliphages and \textit{Clostridium perfringens} may be used concurrently in monitoring source water, microbial removal efficiency of wastewater treatment plants and monitoring health effects (Riesbro et al., 2012).
2.3 Need for Microbial Water Quality Monitoring

Fecal contamination of drinking water is one of the main causes of diarrheal illness in low-resource settings (WHO/UNICEF, 2009). Many of these cases of illness could be avoided if regular microbial water quality testing were performed to determine the microbial quality of drinking water, which could prompt action to remediate fecally contaminated water when found. These tests results can inform communities or households of whether their current drinking water source is safe, if they should seek other sources of drinking water, or use water treatments such as disinfection treatment before consumption. The same is true for situations following natural disasters in which water infrastructure may be impaired and thus drinking water quality may be compromised. There exist many tests to detect and quantify *E. coli* and other fecal coliforms in drinking water; however the tests may be complex, time-consuming, and expensive (Bain et al., 2012a). Current methods may not be appropriate for low-resource settings. However, the Compartment Bag Test (CBT) offers an alternative to the other tests that enable its use in these settings (Bain et al., 2012a; McMahan et al., 2011; Stauber et al., 2014).

2.4 Current Microbial Water Quality Tests

The three current EPA-approved standard methods for detecting *E. coli* in drinking water sources are membrane filtration using differential and selective chromogenic and fluorogenic agar media such as Bio-Rad RAPID’*E. coli* 2™ agar (Bio-Rad) or BD MI agar (MI), multiple tube fermentation (MTF) tests using differential and selective broth culture media, and chromogenic and fluorogenic defined substrate tests, such as IDEXX Colilert® Quanti-Tray 2000 (Colilert) (Edberg et al., 2000; Rompre et al., 2002). Other EPA-approved chromogenic substrate tests that are used less frequently, due to their smaller sample volumes analyzed are
pour plate methods, including Coliscan™ Easygel® (Easygel). All of these tests require resources and conditions that are not readily available in most resource-poor settings, such as in rural areas of developing countries (WHO, 2011; US EPA, 2002). While these methods are appropriate for testing water in developed countries where trained personnel, electricity, and expensive laboratory equipment are available, most rural regions of developing countries will not have access to any of these requirements (Bain et al., 2012a). Therefore, these current microbial water quality tests will not be available to accurately assess the quality of drinking water in these settings.

The membrane filtration method enumerates *E. coli* colonies on agar medium within 24 hours (Dufour et al., 1981; Edberg et al., 1988, US EPA, 2002). This method involves filtering up to 100 mL of sample water through a 0.45 µm pore size membrane filter using a filter funnel and a source of vacuum, such as a side-arm filter flask with vacuum suction. The vacuum suction pulls the sample water through the membrane, leaving the *E. coli* bacteria evenly distributed across the membrane filter surface. The membrane filter is then transferred from the filter funnel to an agar medium using sterile forceps (for example, dipped in 70% ethanol and passed through a flame). The agar media used such as Bio-Rad RAPID’*E. coli* 2™ agar (Bio-Rad) or BD MI agar (MI) (US EPA, 2002) are differential and selective for the growth of *E. coli* and other coliform bacteria. The agar medium plate containing the membrane filter is then incubated at 44.5°C for 18-24 hours. Isolated *E. coli* colonies produce a distinctive visible color or they fluoresce upon exposure to long-wave UV light. The limit of detection can be great, depending upon volume of sample water analyzed, the extent of sample dilution, and the size (area) of membrane filter, allowing for high numbers of colonies to be counted. This method requires several expensive elements: various pieces of laboratory equipment such as the filter assembly
and a source of vacuum, electricity (for a vacuum pump and bacteriological incubator), and trained personnel able to carry out the steps of the analytical method and score the results.

The multiple tube fermentation (MTF) technique is a quantal method that gives a most-probable-number (MPN) estimation of *E. coli* concentration in water samples. The MTF method utilizes differential and selective liquid broth media and multiple sample volumes that are scored as positive or negative for the distinctive growth of *E. coli*, such as the appearance of fluorescence under long wavelength UV light using fluorogenic EC-MUG medium (Edberg et al. 1988). This method uses several culture tubes requiring precise measurements using pipets, racks to hold the culture tubes, and other sterile laboratory equipment. Positive results take 48 hours and have lower precision than methods based on enumerating colonies, such as membrane filtration, though this technique is sensitive (US EPA, 1986). It is a tedious procedure requiring the preparation of sterile broth culture media usually by autoclaving, trained personnel, electricity, and expensive laboratory equipment. Thus, the MTF method is not an appropriate or practical method for use in resource-poor settings.

The IDEXX Colilert® Quanti-Tray 2000 (Colilert) method is widely used in the U.S. and many other countries for quantifying *E. coli* in water samples as a MPN. This method uses multi-well trays, called IDEXX Quanti-Trays and 100 mL bottles, a chromogenic and fluorogenic medium (Colilert), and an IDEXX Quanti-Tray sealer, all of which are expensive (for example, the sealer alone costs $4000), and the sealer requires an electrical power source (IDEXX, 2014). Water samples are poured into the bottles in 100 mL volumes and the Colilert defined substrate reagent medium is added and shaken until dissolved. The sample is then poured into a Quanti-Tray and run through the sealer and the tray is then incubated at 36 °C or 44.5 °C for 24 hours. The results for *E. coli* are read as blue fluorescence in the wells of the tray using long-wave UV
light and the concentration of *E. coli* is expressed as an MPN value per 100 mL sample based on the number of positive and negative wells. Quanti-Tray 2000 can enumerate from 1-2,419 MPN, giving a wide range of concentration values. While simpler than the previous two methods, this method is the most expensive on account of the required purchase of a Quanti-Tray sealer and the relatively high cost of the Colilert defined substrate culture medium.

Coliscan™ Easygel® (Easygel) uses only a 5 mL sample of water, a bottle containing the Easygel medium, and a proprietary petri dish that has been coated with a chemical catalyst that solidifies the pectin gelling agent in the culture medium. The sample water is mixed in the bottle with the Easygel medium, poured into a pre-treated petri dish, and allowed to solidify for 40 minutes. The petri dish is then incubated at 44.5°C for 24 hours (Coliscan Easygel, 2014). While simple, this method still requires trained personnel, laboratory equipment, electricity, and frozen storage of the proprietary culture medium. Furthermore, the small sample size limits the lower detection limit for *E. coli* to 20 or more CFU per 100 mL, which is well above the level of <1 *E. coli* per 100 mL that is considered safe for drinking water.

### 2.5 Limitations of Current Microbial Water Quality Tests in Low Resource Settings

Currently approved methods for the quantification of *E. coli* in drinking water samples require the use of specialized equipment, including an electrically powered incubator, and entail complicated procedures that must be performed by trained personnel (Bain et al., 2012a). Additionally, some methods necessitate the preparation and sterilization of culture media and water sample vessels or require frozen or cold storage of perishable bacteriological media. Many areas of the world that lack access to improved drinking water sources are located in remote rural regions where little or nothing is known about the microbial quality of drinking water sources.
used by communities and households. In such low-resource settings, which may also be very isolated, accessible methods for determining the quality of drinking water sources are lacking. Furthermore, standard methods used to monitor microbial water quality for regulatory compliance in even developed countries may be extremely difficult to use in these types of settings (Bain et al., 2012a). For example, the US EPA procedures for microbial analysis of water samples state that the sample must be kept between 1-4°C during transit and should be analyzed as soon as possible after collection and not more than 30 hours post-collection (US EPA, 1982). It is difficult to achieve these sample-handling conditions in low-resource settings and the quality of analysis of a sample that does not follow these guidelines will potentially be compromised. Therefore, there is a need for an accessible, low-cost, portable, simple method that does not require specialized and highly skilled analysts, additional equipment and materials, such as an incubator, and can be performed on-site, to determine the microbial quality of drinking water in low-resource settings (Bain et al., 2012a; Onda et al., 2012; Sundram et al., 2000).

2.6 How the Compartment Bag Test Overcomes Limitations in Low Resource Settings

The Compartment Bag Test (CBT) is a novel method for quantifying *E. coli* in drinking water samples and has the potential to overcome the barriers to microbial water quality testing in low resource settings (Bain et al., 2012a; McMahan et al., 2009; 2011). The CBT is portable, self-contained, simple to perform with few steps, can be visually scored, requires no cold chain or supporting equipment and specialized materials, and can be performed on-site (Stauber et al., 2014). The CBT is a polyethylene bag (Whirl-Pack, Nasco) that was modified to provide separate internal compartments or chambers of 56, 30, 10, 3, and 1 mL sample volumes, totaling 100 mL. A Hi-*E. coli* test bud of *E. coli* bacteriological medium (HiMedia Labs, Mumbai, India)
containing a chromogenic glucuronide substrate, 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-gluc), is added to a water sample and swirled to fully dissolve (Manafi, 1996). Once the medium reagent is dissolved, the sample with dissolved medium is transferred to a sterile CBT. The sample is then distributed among the 5 compartments of the CBT by tilting the bag from side to side and manual adjustment (squeezing) of the compartment volumes. An external 2-piece plastic spring clip is placed across the bag above the liquid levels in the compartments but below the tops of the compartments to isolate the compartments from each other. The sealed bag is then incubated at 27°C to 44.5°C for 18-24 hours (or up to 48 hours when temperatures are in the 25°C to 30 °C range) and the compartments that show bacterial growth with any trace of blue or blue-green color are considered to be positive for *E. coli* growth. The combination of positive compartments and their volumes are converted to an MPN estimate of the *E. coli* concentration per 100 mL of water.

Because of the lightweight, plastic components of the CBT, the test is portable and compact. There are no glass components or heavy and bulky equipment needed for the CBT, allowing the test to be robust in use. The CBT can be incubated at reasonable ambient incubation temperatures, between 27°C to 44.5°C. In tropical regions with a warm climate, an incubator may not be necessarily. The use of the chromogenic medium allows for the CBT end point result can be visually scored without the need for a UV lamp. There are few steps to the CBT method. A chlorine tablet can be added to disinfect the used CBT, and the test can be disposed of as normal trash. Table 2.2 compares the CBT to some current *E. coli* tests based on key performance criteria. The CBT is designed for testing drinking waters with contamination levels within the range of 0 to 100 *E. coli* MPN/100 mL, and can be used even with turbid waters.
Table 2.2 Comparison of the CBT to current drinking water tests

<table>
<thead>
<tr>
<th>Sample Volume</th>
<th>MTF</th>
<th>MF</th>
<th>QT</th>
<th>EG</th>
<th>CBT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Several volumes, 100 mL total</td>
<td>1 mL, 10 mL, 100 mL</td>
<td>100 mL</td>
<td>1 mL or 5 mL</td>
<td>Several different volumes, 100 mL total</td>
</tr>
<tr>
<td>Quantification Method</td>
<td>MPN</td>
<td>Colony count</td>
<td>MPN</td>
<td>Colony count</td>
<td>MPN</td>
</tr>
<tr>
<td>Electricity need</td>
<td>Yes (incubator)</td>
<td>Yes (incubator)</td>
<td>Yes (Plate sealer and incubator)</td>
<td>Yes (incubator)</td>
<td>No</td>
</tr>
<tr>
<td>Supplemental equipment</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td># of Steps</td>
<td>Multiple</td>
<td>Multiple</td>
<td>4 Steps</td>
<td>4 Steps</td>
<td>4 Steps</td>
</tr>
<tr>
<td>Portable</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Robust</td>
<td>No</td>
<td>No</td>
<td>Somewhat</td>
<td>Somewhat</td>
<td>Somewhat</td>
</tr>
<tr>
<td>Compact</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Somewhat</td>
<td>Yes</td>
</tr>
<tr>
<td>Readily detectable endpoint</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Rapid results</td>
<td>up to 48 Hrs</td>
<td>24 Hrs</td>
<td>18-24 Hrs</td>
<td>24 Hrs</td>
<td>24-48 Hrs</td>
</tr>
<tr>
<td>Range of results</td>
<td>Low to High</td>
<td>Low to High</td>
<td>Low to High</td>
<td>Mid to High</td>
<td>Low to Mid</td>
</tr>
<tr>
<td>Chromogen vs. fluorogen</td>
<td>Both</td>
<td>Chromogen</td>
<td>Both (Fluorogen for E. coli)</td>
<td>Chromogen</td>
<td>Chromogen</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes+</td>
</tr>
<tr>
<td>Specificity</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Not as good</td>
<td>Yes+</td>
</tr>
<tr>
<td>Precision</td>
<td>Yes (Moderate)</td>
<td>Yes</td>
<td>Yes</td>
<td>Maybe</td>
<td>Moderate~</td>
</tr>
<tr>
<td>Works at ambient temp</td>
<td>Maybe but not recommended</td>
<td>Maybe but not recommended</td>
<td>Yes (but not recommended)</td>
<td>Maybe but not recommended</td>
<td>Yes</td>
</tr>
<tr>
<td>Unit cost (not including cost of incubator)</td>
<td>High due to labor and preparation</td>
<td>High due to labor and preparation</td>
<td>$5.50 per sample (plus Sealer, $4000)*</td>
<td>$2.20 per sample*</td>
<td>$5-10 per sample**</td>
</tr>
</tbody>
</table>

* (Bain et al., 2012a), ** (Pricing depends on quantity, Aquagenx, 2012), ~ (Stauber et al., 2014)
(Modified from Pierson, K.A., 2010)
2.7 Previous Compartment Bag Test Performance Evaluations

Comparing the CBT to Idexx Quanti-Tray Colilert method

Prior laboratory performance studies were conducted by McMahan et al, to characterize the ability of the CBT to quantify *E. coli* in drinking water by comparing it to the IDEXX Colilert® Quanti-Tray 2000 (QT), a recognized standard method for microbial water quality testing (McMahan et al, manuscript in preparation). A total of 884 surface water samples were collected over eleven sampling dates from surface water sites in Chapel Hill, North Carolina (United States), supplemented with specific growth medium for *E. coli*, either Colilert for QT or lauryl tryptose broth (LTB) supplemented with X-gluc for CBT, and incubated for 24 to 48 hours at three different incubation temperatures, 44.5°C, 37°C, or 27°C.

The comparison between the QT and CBT analytical methods using the Mann-Whitney test in Figure 2.1 and the Bayesian hierarchical analysis in Figure 2.2 both indicate that the new CBT procedure yields results consistent with those of the Colilert QT procedure. This assessment is based, in part, on the frequency with which the confidence intervals for the difference between the *E. coli* median values derived from the two testing procedures contain zero. Because these intervals are expected to contain zero roughly 95% of the time, it is expected that of 30 intervals, between 24 to 30 of them contain zero. More specifically, of the 30 confidence intervals constructed (excluding trial 6) for the difference between the QT and CBT results for each statistical procedure, 24 from the Bayesian analysis include zero, 16 from the conventional analysis excluding right-censored data include zero, and 28 from the conventional analysis including right-censored data include zero. Because two of the three analytical comparison procedures (the Bayesian analysis, and the MPN method including right-censored data) appear to provide an adequate explanation for the observed
differences, there appears to be sufficient statistical evidence to reject the hypothesis that the procedures are different.

Results recorded at incubation periods of both 24 and 48 hours indicate that the CBT yields results consistent with those from the Colilert QT system for E. coli detection, and that the CBT samples incubated at non-standard temperatures (temperatures above 25°C and below 37°C) gave results equivalent to standard temperatures. Therefore, ambient temperature incubation is possible and the CBT has the potential to serve as a low-cost, accessible solution for practical microbial drinking water quality testing in low resource settings.

Figure 2.1 Water quality sample analysis results for E. coli for each combination of sampling trial, incubation temperature, and incubation period, including Bayesian and conventional (MPN-derived) medians using QT (top panel) and CBT (bottom panel). Bayesian credible intervals which end in an arrow (see trial 6, CBT results) indicate that the interval bound is several orders of magnitude greater than the panel y-axis limits. Note that y-axes in both panels are on a logarithmic scale.
Figure 2.2 Difference between QT and CBT results, measured as the difference between the assessed \textit{E. coli} concentration median for each combination of sampling trial and incubation temperature. Bayesian credible intervals which end in an arrow (trial 6) indicate that the interval bound is several orders of magnitude less than the panel y-axis limits.

Results from culture-based confirmatory identification of \textit{E. coli} bacteria isolated from positive CBT compartments after incubation were further obtained in diagnostic studies to screen for presumptive \textit{E. coli} in both positive and negative compartments by streaking initially from wells of the QT and compartments of the CBT for \textit{E. coli} isolation on Bio-Rad’\textit{E. coli 2}\textsuperscript{TM} agar medium and observing for characteristically colored \textit{E. coli} colonies. Table 2.3 lists the calculated identification statistics from these confirmatory analysis results. Based on the appearance of characteristic colonies on streaked plates of the Bio-Rad chromogenic \textit{E. coli} agar medium, \textit{E. coli} presence was confirmed in all color positive compartments of plastic bags. Surprisingly, however, \textit{E. coli} presence was also found in 27\% of color negative compartments of plastic bags.
Table 2.3 Summary statistics for *E. coli* detection in presumptive positive and negative CBT compartments by streak plate isolation on a chromogenic *E. coli* agar medium

<table>
<thead>
<tr>
<th></th>
<th>24 Hours</th>
<th>48 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>73%</td>
<td>82%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>PPV</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>NPV</td>
<td>68%</td>
<td>66%</td>
</tr>
<tr>
<td>False Positive Rate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>False Negative Rate</td>
<td>27%</td>
<td>18%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>71%</td>
<td>79%</td>
</tr>
</tbody>
</table>

Sensitivity refers to how well a test correctly identifies true positives. Therefore, in this study, sensitivity indicates how well the CBT can perform at detecting low concentrations of *E. coli* (e.g., an *E. coli* MPN of 1 or several per 100mL). As shown in Table 2.3, the sensitivity of the CBT increased from 73% after 24 hours incubation to 82% after 48 hours incubation, indicating that some *E. coli* present in the water may not be detected by visual observation for the distinctive color of the hydrolysis product of the β-D-glucuronide chromogenic substrate after only 24 hours of incubation. Specificity refers to how well the test correctly identifies true negatives, and the positive predictive value (PPV) is a measure of the proportion of positive compartments that are correctly identified, which is also a measure of the precision of the test. This study found very high specificity and PPV for the CBTs as shown in Table 2.3 with values of 100% for both after 24 and 48 hours of incubation. The negative predictive value (NPV) is a measure of the proportion of negative compartments that are correctly identified. At 24 hours, less than a third (32%) of the negative compartments tested were positive for *E. coli*. At 48 hours, one of the negative compartments had changed color to become visually positive, but 34% of the color negative compartments remained positive for *E. coli* based on isolation of characteristic *E. coli* colonies on a differential and selective chromogenic agar medium.
2.8 Improving Compartment Bag Test bacteriological culture

The X-gluc chromogen has been shown previously to be highly accurate in detecting *E. coli* based on its detection of ß-glucuronidase activity, with a 1% false-negative rate and 5% false-positive rate (Watkins et al., 1988). However, in the McMahan et al. study, the CBT demonstrated a false positive rate of 0% and false-negative rate of 28-37%, after 24 and 48 hours of incubation, respectively, via identification of isolated bacteria by biochemical assay using the Enterotube™ II system (Becton Dickinson) for identification of *Enterobacteriaceae* (McMahan et al., manuscript in preparation). In order to not underestimate *E. coli* concentration in water, due to implications for public health protection, further evaluation is needed to better determine the accuracy, sensitivity, and specificity of the CBT.

In this previous study, the CBT medium used for comparison with Colilert was lauryl tryptose broth (LTB) medium, a standard coliform medium, supplemented with 0.1114 g/L of X-gluc, a chromogenic Beta-D-glucuronide substrate. The efficacy of LTB in culturing *E. coli* is well established (Feng & Hartman, 1992; Park et al., 1995), and the results found in this study show that this efficacy was still apparent with the addition of the chromogenic substrate X-gluc to LTB. However, some limitations of this medium could be that due to varying nutritional requirements and the presence of the anionic surfactant sodium lauryl sulfate, certain bacterial strains and injured bacteria may grow poorly or fail to grow. Also, LTB may form a precipitate when stored at colder temperatures. Although this precipitate generally dissipates upon warming to room temperature, precipitation of medium may hinder visible detection of *E. coli* growth in a liquid medium. Therefore, an improved chromogenic medium for *E. coli* growth was developed.

The new medium proposed for evaluation in this research, Hi-*E. coli*, utilizes different and multiple compounds to provide the source for nitrogen, vitamins, amino acids, fermentable
carbohydrates, tryptophane (a substrate for indole production), a chemical agent to resuscitate injured cells, and a synthetic analog of lactose that inactivates the \textit{lac} repressor and induces synthesis of beta-galactosidase. The concentration of X-gluc is about 163\% greater in the new medium than in the previously studied medium mixture. Table 2.4 below compares the previously used LTB supplemented with X-gluc and Hi-\textit{E. coli} bacteriological medium composition. An increase in X-gluc concentration may allow for sufficient concentration of the hydrolysis product from the chromogenic glucuronide substrate by the \textit{E. coli} bacteria growth to produce a visible color change for detection of \textit{E. coli} with decreased occurrence of previous false negatives.

<table>
<thead>
<tr>
<th>Purpose of Ingredients</th>
<th>LTB with X-gluc</th>
<th>Hi-\textit{E. coli}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ingredients</td>
<td>Grams/Liter</td>
</tr>
<tr>
<td>Provide nitrogen,</td>
<td>Tryptose</td>
<td>20.0</td>
</tr>
<tr>
<td>vitamins, amino</td>
<td>Lactose</td>
<td>5.00</td>
</tr>
<tr>
<td>acids, &amp; fermentable</td>
<td>Sodium Pyruvate</td>
<td>1.00</td>
</tr>
<tr>
<td>carbohydrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maintain osmotic</td>
<td>Sodium Chloride</td>
<td>5.00</td>
</tr>
<tr>
<td>balance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffering agents</td>
<td>Monopotassium Phosphate</td>
<td>2.75</td>
</tr>
<tr>
<td></td>
<td>Disodium Phosphate</td>
<td>2.75</td>
</tr>
<tr>
<td>Inhibit non-</td>
<td>Sodium Lauryl Sulfate</td>
<td>0.10</td>
</tr>
<tr>
<td>coliform bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detection of \textit{E.</td>
<td>X-gluc</td>
<td>0.11</td>
</tr>
</tbody>
</table>
2.9 Compartment Bag Test performance evaluations with improved bacteriological medium

Comparing the CBT to membrane filtration for *E. coli* detection in water

In a recent study by Stauber et al. (2014), 261 water samples collected around metro-Atlanta in volumes of 10, 50, or 100 ml were analyzed in duplicate by membrane filtration with a selective medium (mI agar, Becton Dickinson, Sparks, MD) containing chromogenic and fluorogenic β- glucuronide and β-galactoside substrates for the detection and enumeration of *E. coli* and coliforms, respectively, following standard method 1604 (US EPA, 2002). After applying the membranes of filtered water to the agar medium, the plates were inverted and incubated for 18–24 h at 37°C. *E. coli* colonies were quantified and reported as colony forming units (CFU) per 100 mL (US EPA, 2002). The same water samples were also processed in duplicate in volumes of 100 mL by the CBT method with the improved Hi-*E. coli* medium. Positive compartments of the bag were identified as those that turned a blue-green color, indicating the presence of *E. coli* due to the hydrolysis of the chromogenic β-glucuronide substrate. The categorical results are shown in Table 2.5 and were also compared on the basis of sensitivity [(true positives) / (true positives + false negatives)] and specificity [(true negatives) / (true negatives + false positives)] for the presence of *E. coli*. The performance characteristics of the CBT for detection of *E. coli* compared with membrane filtration are shown in Table 2.6. Stauber et al found the CBT to have an accuracy of 95% and a decreased false negative rate at about 5% (Stauber et al., 2014).
Table 2.5 Comparison of categorical concentrations of *E. coli* from the CBT and membrane filtration for indicated numbers of samples and percentage of samples per category

<table>
<thead>
<tr>
<th>CBT (MPN/100 ml)</th>
<th>Membrane Filtration (CFU/100 ml)</th>
<th>&lt;1</th>
<th>1-10</th>
<th>11-100</th>
<th>&gt;100</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td></td>
<td>28</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>70.0%</strong></td>
<td><strong>20.0%</strong></td>
<td><strong>10.0%</strong></td>
<td><strong>0%</strong></td>
<td><strong>100%</strong></td>
</tr>
<tr>
<td>1-10</td>
<td></td>
<td>1</td>
<td>30</td>
<td>9</td>
<td>2</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>2.38%</strong></td>
<td><strong>71.4%</strong></td>
<td><strong>21.4%</strong></td>
<td><strong>4.76%</strong></td>
<td><strong>100%</strong></td>
</tr>
<tr>
<td>11-100</td>
<td></td>
<td>0</td>
<td>7</td>
<td>50</td>
<td>11</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>0%</strong></td>
<td><strong>10.3%</strong></td>
<td><strong>73.5%</strong></td>
<td><strong>16.2%</strong></td>
<td><strong>100%</strong></td>
</tr>
<tr>
<td>&gt;100</td>
<td></td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>103</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>0%</strong></td>
<td><strong>0%</strong></td>
<td><strong>7.21%</strong></td>
<td><strong>92.8%</strong></td>
<td><strong>100%</strong></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>29</td>
<td>45</td>
<td>71</td>
<td>116</td>
<td>261</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>11.1%</strong></td>
<td><strong>17.2%</strong></td>
<td><strong>27.2%</strong></td>
<td><strong>44.4%</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

(Modified from Stauber et al., 2014)

Table 2.6 Performance characteristics of the CBT for the detection of *E. coli* compared to membrane filtration

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>94.9%</td>
</tr>
<tr>
<td>Specificity</td>
<td>96.6%</td>
</tr>
<tr>
<td>PPV</td>
<td>99.6%</td>
</tr>
<tr>
<td>NPV</td>
<td>70%</td>
</tr>
<tr>
<td>False Positive Rate</td>
<td>3.4%</td>
</tr>
<tr>
<td>False Negative Rate</td>
<td>5.1%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>95%</td>
</tr>
</tbody>
</table>

(modified from Stauber et al., 2014)

2.10 Need for further CBT evaluation

The consumption of fecally contaminated water is one of the major causes of the more than 1.8 million deaths annually due to diarrheal disease (WHO/UNICEF, 2009). More available and accessible water quality testing could be a transformative catalyst for interventions and policy change to improve access to clean water and diminish severe health consequences of waterborne disease. The CBT is designed as a field test for detecting and quantifying *E. coli* in drinking water samples, and overcomes obstacles that hinder the applicability of current methods.
for low-resource settings (Bain et al., 2012a; McMahan et al., 2011; Stauber et al., 2014). The simplicity of the test allows anyone with brief training to test their own water, thereby empowering people with knowing if their water is safe so that they can determine their own remedial actions. There are several different applications of this technology for water quality analysis including management, surveillance and verification of water quality, food and beverage safety, and use in disaster preparedness. Previous research on the CBT by McMahan et al. indicated that the CBT yields consistent results when compared to the QT system with Colilert medium for *E. coli* detection, and that the CBT samples incubated at non-standard temperatures gave similar results to those at standard temperatures (McMahan et al., manuscript in preparation). However the sensitivity and accuracy ranged from 71-82%. The study conducted by Stauber et al. indicated that the CBT yields consistent results when compared to membrane filtration for *E. coli* detection (Stauber et al., 2014). This study also demonstrated that the use of the improved Hi-*E. coli* medium can increase the accuracy of the CBT for detection and quantification of *E. coli* by 20.3-33.8%.

Based on the limited available literature, the new CBT media should be further tested in the laboratory and in the field. While previous laboratory based experiments on the CBT have been conducted, there is the potential for performance differences between laboratory effectiveness and efficacy in the field. The efficacy and applicability of the CBT method for use in low-resource settings should be compared to other currently available and accepted methods for *E. coli* analysis of water. Evaluation of CBT in the field and documentation of observations and experiences in its use and effectiveness will significantly inform the challenges of microbial field testing and identify possible solutions to scientific, technical, adaptability, acceptability, and administrative problems facing the CBT test. Furthermore, the water quality results from the
inclusion of CBT in household surveys can better depict the status of progress in achieving of the Millennium Development Goals on drinking water access, substantiate the Joint Monitoring Program’s proposed water quality target for the post-2015 Sustainable Development Goals (SDGs) which includes measurement of E. coli concentrations as a fecal indicator of drinking water, and provide quality actionable data for local information, action, interventions, and national policy status and directions.
CHAPTER 3: LABORATORY EVALUATION OF THE COMPARTMENT BAG TEST

3.1 Introduction

The Joint Monitoring Programme of the UN relies greatly on household surveys to determine the safety of the drinking water supply present in the home. Due to the unavailability of simple, convenient, and affordable methods to test water in the field, a classification system for improved and unimproved drinking water sources is used as a proxy to identify safe and unsafe household water in lieu of physically testing microbial water quality (WHO/UNICEF, 2012). A novel water quality field test was developed by the investigators of this study to overcome the obstacles of microbial water quality testing in low resource settings. The method is called the Compartment Bag Test (CBT) and the test quantifies *Escherichia coli* in 100-mL drinking water samples. The CBT has the potential to overcome the barriers to microbial water quality testing in low resource settings because it is portable, simple to perform with few steps, can be visually scored, requires no cold chain, supporting equipment, or specialized materials, and can be performed on-site (McMahan et al, 2009; 2011; Bain et al., 2012a; Stauber et al, 2014).

The CBT consists of a clear, sterile polyethylene bag (Whirl-Pack, Nasco, USA) that was modified to provide separate internal chambers of 56, 30, 10, 3, and 1 mL sample volumes, totaling 100 mL. An *E. coli* bacteriological medium containing a chromogenic glucuronide substrate, 5-bromo-4-chloro-3-indolyl- ß -D-glucuronic acid (X-gluc), is added to a water sample and swirled to fully dissolve (Watkins et al., 1988). The original *E. coli* medium for the CBT was
lauryl tryptose broth with added X-Gluc. Once the medium reagent is dissolved, the sample is transferred to a sterile CBT. The sample is then distributed among the five compartments to their fill lines by tilting the bag from side to side while manually adjusting the compartment volumes by squeezing the bag exterior. An external 2-piece spring plastic clip is placed across the bag above the liquid levels in the compartments but below the tops of the compartments to isolate the compartments from each other. The sealed bag is then incubated at 27°C-44.5°C for 18-24 hours, or up to 48 hours when temperatures are below 30 °C, and the compartments that show bacterial growth and any trace of blue or blue-green color from the hydrolysis product of the X-Gluc are considered to be positive for _E. coli_ growth. Ambient incubation temperatures for _E. coli_ growth are possible and their effective performance has been demonstrated for several different fecal indicator bacteria tests in a previous study (Brown et al., 2011). The combination of positive and negative compartments and their volumes are scored and then expressed as a Most Probable Number (MPN) estimate of the _E. coli_ concentration per 100 mL of water based on a table developed for and provided with the test.

The purpose of this research is to evaluate the performance of an updated version of the CBT for detecting and quantifying _E. coli_ in samples of potential drinking water sources using an improved culture medium (Hi- _E. coli_ test bud, HiMedia Labs, Mumbai, India). Field samples of ambient water were analyzed in the laboratory to further characterize the performance of the test with the new chromogenic medium for improved detection and quantification of _E. coli_ compared to a standard _E. coli_ fluorogenic, defined substrate MPN medium, Colilert (IDEXX), with sample incubation at temperatures of 27, 37 and 44.5 °C. Bacteria were isolated from CBT compartments after incubation for further characterization. Presumptive _E. coli_ colonies isolated from bag compartments, that were scored both positive and negative based on color change, were
culture purified and then speciated to determine if the CBT method accurately detected \textit{E. coli}. Speciation was determined by biochemical analysis using the \textit{Enteroplturi} system (Becton, Dickinson and Company, USA), and by molecular analysis using qPCR for the \textit{uidA} gene (Biogx, Inc, USA), as well as matrix-assisted laser desorption/ionization - time of flight mass spectrometry (MALDI-TOF MS) analysis (bioMérieux, Inc, France) (Bej et al, 1991; Holland et al, 1999).

\textbf{3.2 Methods}

Surface waters (with turbidity between 3-8 NTU) from Morgan Creek in Chapel Hill, North Carolina were analyzed by the CBT using the CBT Hi-\textit{E. coli} chromogenic medium and Colilert \textit{E. coli} fluorogenic medium with incubation at three different temperatures: 27\(^\circ\)C, 37\(^\circ\)C, and 44.5\(^\circ\)C. Both media detect \textit{E. coli} based on hydrolysis of a \(\beta\)-D-glucuronide substrate to yield a visibly detectable hydrolysis product. Five separate experiments with varied sample water dilutions were conducted that produced 600 media comparison samples, half conducted with Hi-\textit{E. coli} medium and half with Colilert medium.

The 600 samples of water in this dataset were evenly divided into one group analyzed with the CBT and Hi-\textit{E. coli} medium and another group analyzed with the CBT and Colilert medium. For both analysis groups reported \textit{E. coli} concentration were reported as MPN/100 mL. Each water sample was prepared by mixing ambient surface water with deionized water to create one of two different dilution levels: a 1:10 dilution by combining 10 mL of ambient surface stream water with 90 mL of sterile diluent and a 1:2 dilution by combining 50 mL of ambient surface stream water with 50 mL of sterile diluent. The sterile diluent was autoclaved deionized water. Within each test group, 180 samples were made at the 1:2 dilution and 120 samples were
made at the 1:10 dilution. For each test group and within each dilution level, the incubation temperatures of the water samples were evenly divided between 27°C, 37°C, and 44°C, so that each incubation temperature-sample dilution combination for the 1:10 dilution had a sample size of 40 and the 1:2 dilution had a sample size of 100. Table 3.2 presents how samples were split between the temperature and dilution levels for the CBT test group, and the Colilert test samples were divided in the same way.

Table 3.1 Numbers of water samples tested with the CBT at each incubation temperature for each dilution level in either Colilert medium or CBT Hi-E. coli medium

<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>Incubation Temperature 27°C</th>
<th>Incubation Temperature 37°C</th>
<th>Incubation Temperature 44°C</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>120</td>
</tr>
<tr>
<td>1:2</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>180</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>300</td>
</tr>
</tbody>
</table>

Bacteriological medium, either CBT Hi-E. coli or Colilert, was added to a 100 mL water sample per manufacturer’s instruction and swirled to fully dissolve. Once the medium was dissolved, the sample with dissolved medium was poured into a sterile CBT. The sample was then distributed among the 5 compartments to their fill lines by manual adjustment of the compartment volumes. The CBT was then sealed with an external 2-piece spring plastic clip across the bag above the liquid levels in the compartments but below the tops of the compartments to isolate the separate compartments. The sealed bag was incubated at 27°C, 37°C or 44.5°C for 18-24 hours. The compartments that contained any trace of blue or blue-green color were scored positive for *E. coli* growth for Hi-E. coli medium. The compartments that contained any trace of yellow color plus blue fluoresce under long wave UV light were scored positive for *E. coli* growth for the Colilert medium. The combination of positive and negative
compartments and their volumes were used find the MPN estimate of the \emph{E. coli} concentration per 100 mL of water based on a table of previously calculated MPN values for all combinations of positive and negative compartment volumes in the CBT.

Of the 300 samples analyzed using Hi-\emph{E. coli} medium, a total of 559 bacteria were isolated and purified from positive and negative chambers by using sterile wooden sticks for culture purification via the steps in Table 3.1 below. Purified bacteria isolates were stored as frozen cultures. They were later thawed, re-plated, and re-grown before biochemical and molecular analysis by streak plating on non-selective tryptic soy agar (TSA) plates with incubation at $44.5^\circ C$ for 18-24 hours to obtain isolated colonies. The biochemical assay involved picking material from an isolated colony with the self-contained inoculating wire of the \emph{EnteroPlurri} test. The molecular analysis was done by two methods: qPCR targeting the \textit{uidA} gene for glucuronidase activity, and matrix-assisted laser desorption/ionization - time of flight mass spectrometry (MALDI-TOF MS) analysis.

Table 3.2 Summary of triple-streak procedure for isolating and culturing \emph{E. coli} and re-growing frozen cultures for further biochemical and molecular assays for speciation

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Medium</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1\textsuperscript{st} streak</td>
<td>Bio-Rad Rapid'\emph{E. coli} 2 agar</td>
<td>Streaked liquid from a compartment in each CBT onto Bio-Rad Rapid'\emph{E. coli} 2 agar plate (1 plate/CBT)</td>
</tr>
<tr>
<td>2\textsuperscript{nd} streak</td>
<td>Bio-Rad Rapid'\emph{E. coli} 2 agar</td>
<td>Chose isolated colony from first Bio-Rad Rapid'\emph{E. coli} 2 agar plate and re-streaked onto second Bio-Rad Rapid'\emph{E. coli} 2 agar plate, in duplicate (2 plates/CBT)</td>
</tr>
<tr>
<td>3\textsuperscript{rd} streak</td>
<td>TSA</td>
<td>Chose one isolated colony from each Bio-Rad Rapid'\emph{E. coli} 2 agar plate and re-streaked onto TSA agar plate (2 plates/CBT)</td>
</tr>
<tr>
<td>Frozen cultures</td>
<td>TSB + 20% glycerol</td>
<td>Chose one isolated colony from each TSA agar plate and added directly to 1 mL liquid TSB medium in 1.5 mL microcentrifuge tube for freezer storage</td>
</tr>
<tr>
<td>Re-growing</td>
<td>TSA</td>
<td>Streaked liquid from thawed TSB in 1.5 mL</td>
</tr>
</tbody>
</table>
frozen cultures for biochemical and molecular analysis

microcentrifuge tube onto TSA agar for regrowth from frozen culture; isolated colonies picked from TSA plate were used for further assays

The *EnteroPlurri* test (Liofilchem) involved picking a colony from the regrown frozen culture on the TSA plate with the self-contained inoculating wire and inoculating simultaneously all 12 different conventional media compartments and incubating at 37°C for 24 hours. After inoculation and incubation, the 15 biochemical reactions: glucose, gas production from glucose, lysine decarboxylase, ornithine decarboxylase, H₂S, indole, adonitol, lactose, arabinose, sorbitol, Voges-Proskauer, dulcitol, phenylalanine, deaminase, urea and citrate, were be observed in the plastic compartments. The resulting combination of positive reactions as per manufacturer’s instructions, allowed the identification of genus and species within the family of *Enterobacteriaceae* using the key provided by the manufacturer.

For qPCR analysis, a colony from the regrown sample on TSA was picked suspended in 500 µl in the nonselective broth, TSB, and grown overnight in a screw cap tube in order to decrease potential interference of glycerol. From the overnight cultures, 250 µl was centrifuged for 5 minutes (5000 x g), the supernatant was removed with a pipet tip and the pelleted cells retained. In the screw cap tube where the pelleted cells were retained, 100 µl of deionized water was added. The samples were then heated in a boiling water bath for about two minutes in order to lyse the cells. Then, a crude DNA extraction from the approximately 100 µl volume of boiled cell suspension was performed with 1mm silica/zirconium beads (BioSpec, USA) according to the manufacturer’s instructions. *E. coli* analysis via qPCR was then conducted with Scorpion® based chemistry employing lyophilized beads that contained buffer, and primer and probe (Biogx, Inc, USA). This set of forward and reverse primers and probe targets the *uidA* gene that
codes for the enzyme β-glucuronidase, which is responsible for hydrolysis of β-D-glucuronide substrates, such as X-gluc and 4-methyl-umbelliferyl-4-β-D-glucuronide (MUG). Primer and probe sequences are proprietary. A qPCR master mix of lyophilized beads containing the *E. coli*-specific primer and probe set, Omnimix lyophilized beads, and 20 µL reagent grade dilution water were combined with 5 µL of sample and analyzed via the Cepheid Smart Cycler II system: with a thermal cycling regimen of 120 seconds at 95° C, followed by 45 cycles of 5 seconds at 95° C, followed by 43 seconds at 62° C (Noble et al., 2010; Krometis et al., 2011). In order to determine potential qPCR amplification inhibition, SKETA (a salmon sperm DNA target) was also run with about half the samples tested (Biogx, Inc, USA). If a cycle-fluorescence curve came up, the sample was scored as *E. coli* positive; if no cycle-fluorescence curve came up, the sample was scored as *E. coli* negative.

The MALDI-TOF MS method is a soft ionization process and analysis allows for biomolecules, such as DNA and proteins, to be ionized and analyzed (Patel, 2013). A colony from the regrown samples was picked with a sterile toothpick, smeared on a MALDI-TOF MS plate, with a matrix of different samples applied (bioMérieux VITEK MS with version 2.0 Knowledge Base database, bioMerieux, Durham NC). The inoculated plate was irradiated by a laser pulse and allowed for biomolecules to be ionized and desorbed. The sample ions are accelerated in an electric field and enter a flight tube where they are separated based on their differing masses and relative abundance. The mass to charge ratio is measured and a generated spectrum (often referred to as a fingerprint) is compared to a library. MALDI-TOF MS can determine the genus, and often species, by bioinformatics software included with the analytical system in under an hour of analysis time (Croxatto et al, 2011). There was no need to specify the sub-group of bacteria of interest since the database, version 2.0 Knowledge Base, can identify
from a large population of clinically relevant bacteria from the MALDI-TOF MS results. It is often difficult to differentiate *Shigella* species and *E. coli* due to similar properties via MALDI-TOF MS (Ochman et al., 1933). However, this was not an issue in this study because *E. coli* was selected for in samples based on their detection and isolation by β-D-glucuronidase activity using chromogenic culture and isolation media; *Shigella* species are negative for this activity.

In order to evaluate the laboratory performance of the CBT when applied to field samples of ambient surface water, the results from the MPN estimates of CBT with Hi-*E. coli* and Colilert media for different incubation temperatures, as well as the biochemical and molecular speciation results, were compared and statistically analyzed in SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp) and R (R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/). A Chi-Square test was conducted to observe the association between the MPN results of the CBT with its chromogenic medium and the CBT with a standard fluorogenic medium, Colilert, in categories of positivity versus negativity and on the basis of the WHO decimal risk levels for *E. coli* concentrations per 100 mL in the ranges of <0, 1-10, 10-99, and >100 (WHO, 2011).

To further test if the use of the Hi-*E. coli* and Colilert media in the CBT report similar MPN values for *E. coli* concentrations in water samples when both tests are conducted in a laboratory setting, a regression analysis was conducted. First a three-way ANOVA regression with log10(MPN) as the dependent variable and categorical variables for test type, dilution, and temperature was performed. This type of analysis compares the group means of log10(MPN) between each level (i.e. each value) of each categorical variable while controlling for the other the variables. The coefficient of a given level for a given categorical variable measures the effect
on log10(MPN) of that level when compared to a reference level chosen to be the smallest value of the categorical variable. For example, the coefficient on CBT versus Colilert tells us the effect on log10(MPN) of using CBT to measure *E. coli* concentration versus using the Colilert media. In this example, Colilert is the reference level of the categorical variable test type. When coefficients are not statistically significant, we consider them to be no different from 0 so that there is no effect on log10(MPN) of the level under consideration versus the reference level of a given categorical variable.

Similar to the ANOVA regression, logistic regression, which measures the effect of the independent variables on the dependent variable, were also be performed. Three logistic regressions were performed using less than or greater than or equal to 100.0 MPN, less than or greater than or equal to 48.3 MPN, as well as less than or greater than or equal to 1.5 MPN. These three sets of MPN categories are of interest to the study for being at and above the detection limit of the test (left censored versus right censored) as they represent some of the smallest and largest MPN values in the dataset that correspond to very safe and unsafe drinking water, respectively (WHO, 2011). The MPN greater or equal to 48.3 was chosen as a cutoff of interest to the study since it represents the MPN value in the dataset that corresponds to unsafe drinking water and the upper detection limit of the CBT (WHO, 2011; McMahan et al., 2011; Stauber et al., 2014). Because in the logistic case the response is a dummy variable, the coefficients are interpreted as the multiplicative effect on the log odds of having an MPN greater than or equal to the three MPN categories mentioned for a unit change in the explanatory variable, in this case MPN/100 mL, while holding all other variables constant. Taking the exponential of the coefficient provides the multiplicative effect on the odds of having an MPN
greater than or equal to the three MPN categories evaluated, for a MPN/100 mL change in the explanatory variable.

The culture purified presumptive positive isolates from CBT positive chambers and presumptive negative isolates from CBT negative chambers were compared with the results of the biochemical and molecular assays. The results were organized and tabulated in a contingency table. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were calculated.

3.3 Results

Comparison of CBT with Hi- E. coli and Colilert media for E. coli quantification in surface source water samples at different incubation temperatures

The *E. coli* MPN concentrations were reported by three variables including: the culture media used (i.e. Hi-*E. coli* versus Colilert), incubation temperature condition (i.e. 27°C, 37°C or 44.5°C), and dilution level (i.e. 1:10 or 1:2 dilutions). The *E. coli* concentrations are reported as log10(MPN) because normal qq plots of MPN, a common tool used to test the normality of data, suggest that this transformation best mimics the normal distribution and minimizes the skewed pattern of the reported MPN’s. ¹ Because the tests report a finite number of unique values of MPN, gaps exist in the distribution of log10(MPN), therefore a categorical approach to statistical analysis was undertaken. A box and whisker plot of the *E. coli* MPN concentrations for Hi-*E. coli* and Colilert media at different incubation temperatures is shown in Figure 3.1. The number of samples analyzed for each media and incubation temperature combination (i.e. H27, C44) was 100. A scatterplot of paired log10(MPN) results, shown in Figure 3.2, allows for the

¹ Technically log10(MPN+1) was calculated, but for simplicity the transformation will be referred to as log10(MPN).
comparability of *E. coli* detection by Hi-*E. coli* and Colilert media to be better visualized. Again, the number of samples analyzed for each media and incubation temperature combination was 100, so in Figure 3.2 there was 200 samples in each temperature category.

**Figure 3.1** Box and whisker plot of *E. coli* MPN concentrations for Hi-*E. coli* (H) and Colilert (C) media at different incubation temperatures of 27°C, 37°C, and 44.5°C

**Figure 3.2.** Scatterplot of paired media log10(MPN) *E. coli* results as measured by Hi-*E. coli* and Colilert media at different incubation temperatures
To compare categorical \textit{E. coli} MPN results, Chi-Square tests were performed. The null hypothesis for the Chi-Square test states that the row variable is independent of the column variable, or in other words, there is no association between the categorized results of the MPN results using CBT with the Hi-\textit{E. coli} medium or the CBT with the Colilert medium. The Pearson’s Chi-Square value was found to be statistically significant in the cases of 1) Hi-\textit{E. coli} in CBT with \textit{E. coli} MPN results categorized as presence/absence versus Colilert in CBT with \textit{E. coli} MPN results categorized presence/absence across all temperatures evaluated (i.e. 27°C, 37°C or 44.5°C) and 2) Hi-\textit{E. coli} in CBT with \textit{E. coli} MPN results categorized into WHO risk strata categories versus Colilert in CBT with \textit{E. coli} MPN results categorized into WHO risk strata across all temperatures evaluated (i.e. 27°C, 37°C or 44.5°C). Thus the null hypothesis can be rejected and it can be assumed that there is an association between the \textit{E. coli} MPN results of the Hi-\textit{E. coli} medium in the CBT and Colilert medium in the CBT when categorized and compared as listed above. The Pearson’s R values demonstrate that the \textit{E. coli} MPN results of the Hi-\textit{E. coli} in CBT and Colilert in CBT are strongly and positively correlated when categorized by presence/absence and WHO risk strata across all incubation temperatures evaluated. The summary of Pearson’s Chi-Square values and Pearson’s R statistics can be found in Table 3.3.
Table 3.3. Summary of Chi-Square and Pearson’s R correlation statistics for the categorical comparison of Hi-\(E.\ coli\) and Colilert media across different incubation temperatures

<table>
<thead>
<tr>
<th>Categorical Comparison of Hi-(E.\ coli) versus Colilert media</th>
<th>Presence/Absence</th>
<th>X2</th>
<th>df</th>
<th>N</th>
<th>p-value</th>
<th>Pearson's R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>27C</td>
<td>73.446</td>
<td>1</td>
<td>100</td>
<td>&lt;0.0001*</td>
<td>0.857</td>
</tr>
<tr>
<td></td>
<td>37C</td>
<td>77.637</td>
<td>1</td>
<td>100</td>
<td>&lt;0.0001*</td>
<td>0.881</td>
</tr>
<tr>
<td></td>
<td>44C</td>
<td>80.4</td>
<td>1</td>
<td>100</td>
<td>&lt;0.0001*</td>
<td>0.897</td>
</tr>
<tr>
<td>WHO Risk Strata</td>
<td>X2</td>
<td>df</td>
<td>N</td>
<td>p-value</td>
<td>Pearson's R</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27C</td>
<td>219.827</td>
<td>3</td>
<td>100</td>
<td>&lt;0.0001*</td>
<td>0.938</td>
</tr>
<tr>
<td></td>
<td>37C</td>
<td>149.339</td>
<td>3</td>
<td>100</td>
<td>&lt;0.0001*</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>44C</td>
<td>165.061</td>
<td>3</td>
<td>100</td>
<td>&lt;0.0001*</td>
<td>0.892</td>
</tr>
</tbody>
</table>
*Significant at 5% level

To further estimate the effect of variables (i.e. medium used, and incubation temperature) different regressions were performed. As shown in Table 3.4, the ANOVA regression result is that the estimated change in MPN from using Hi-\(E.\ coli\) medium versus Colilert medium is 0 as the p-value on the coefficient is 0.18 and insignificant. This result provides evidence that the Hi-\(E.\ coli\) medium performs similarly to Colilert medium in quantifying \(E.\ coli\) concentration in water samples. As expected, using a dilution of 1:2 (50 mL versus 10 mL) significantly increases the MPN concentration estimate, by 8.67 MPN/100 mL. This intuitively makes sense as more diluted surface waters should have less bacterial contamination and the change is statistically significant. Maintaining a sample at 44° C versus 27° C is associated with a statistically significant increase of 1.22 in MPN/100 mL. Though this change is statistically significant, the magnitude of the effect on MPN is not large. The effect of maintaining samples at 37° C versus 27° C is not statistically significant and the same for both Hi-\(E.\ coli\) medium and Colilert medium.
Table 3.4 Three-way ANOVA regression results with log10(MPN) as the dependent variable.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Estimated change on MPN/100 mL</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBT versus Colilert</td>
<td>-0.09</td>
<td>0</td>
</tr>
<tr>
<td>Dilution 50ml versus 10ml</td>
<td>2.16</td>
<td>8.67</td>
</tr>
<tr>
<td>37° versus 27°</td>
<td>0.10</td>
<td>0</td>
</tr>
<tr>
<td>44° versus 27°</td>
<td>0.20</td>
<td>1.22</td>
</tr>
</tbody>
</table>

*Significant at 5% level

In addition to testing the effect of the explanatory variables on log10(MPN), the variables’ effects on determining if the reported MPN was less than 48.3 or greater than or equal to 48.3 was also measured since this demonstrates that all compartments within the CBT show a positive result. Two other logistic regressions were also performed using less than 100.0 MPN or greater than or equal to 100.0 as well as less than 1.5 MPN or greater than or equal to 1.5, however their results were similar to those in Table 3.4 and are not included here. These three sets of MPN categories are of interest to the study as they represent the smallest and largest MPN values in the dataset that correspond to very safe and unsafe drinking water as well as the upper detection limit of the CBT (WHO, 2011). Through logistic regressions the effect of variables on the odds of having unsafe drinking water can be measured. In Table 3.5 we can see that most of the coefficients are not significant. The odds of having an MPN greater than or equal to 48.3 when the temperature is 44° C are 1.63 times larger than the odds when the temperature is 27° C. All coefficients have p-values greater than 0.05 and are associated with explanatory variables that do not significantly affect the odds of having an MPN greater than or equal to 48.3. Just as in the ANOVA regression, when coefficients are not statistically significant, there is no effect on the log odds of having MPN greater than or equal to 48.3 with a unit change in the independent variable.
Table 3.5 Logistic regression results comparing MPN < 48.3 versus >=48.3 as the dependent variable

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Estimated change in odds of MPN&gt;=48.3</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBT versus Colilert</td>
<td>-0.17</td>
<td>0</td>
</tr>
<tr>
<td>Dilution 50ml versus 10ml</td>
<td>20.03</td>
<td>0</td>
</tr>
<tr>
<td>37° versus 27°</td>
<td>0.34</td>
<td>0</td>
</tr>
<tr>
<td>44° versus 27°</td>
<td>0.49</td>
<td>1.63</td>
</tr>
</tbody>
</table>

*Significant at 5% level

The Chi-Square tests and Pearson’s R values demonstrate that there is an association and the correlation is strong and positive, between the *E. coli* MPN results of the Hi-*E. coli* medium in the CBT and Colilert medium in the CBT when categorized as presence/absence and within WHO risk strata across all incubation temperatures evaluated. Both the ANOVA regression and the logistic regression above suggest that using CBT medium versus Colilert medium does not significantly affect the estimates of *E. coli* MPN concentration per 100 mL. The regressions report no effect of test type or temperature used on log(MPN) nor on the odds of reporting MPN greater than or equal to 100, 48.3, or 1.5. Just as in the ANOVA regression, when coefficients are not statistically significant, there is no effect on the log odds of having MPN greater than or equal to 100, 48.3, or 1.5 with a unit change in the independent variable.

*Further bacteriological analysis of CBT presumptive positive and negative compartments via *E. coli* and coliform isolation by streak-plate colony isolation on a standard *E. coli* and coliform chromogenic agar medium*

A total of 559 bacteria were isolated from positive and negative bag chambers analyzed using the CBT Hi-*E. coli* chromogenic broth culture medium. Of this total, 411 presumptive positive samples were isolated from CBT color positive bag chambers, and 148 presumptive
negative samples were isolated from CBT chambers that were color negative using the Hi-\textit{E. coli} medium. These isolates were obtained by streaking onto plates of the differential and selective \textit{E. coli} and coliform agar medium Rapid’\textit{E. coli} 2 (BIO-RAD). The results of presumptive \textit{E. coli} bacteria colony isolation from color positive and color negative bag compartments are recorded in Table 3.6 below.

According to presumptive \textit{E. coli} colony confirmation via streak-plating onto this chromogenic Rapid’\textit{E. coli} 2 agar medium, the CBT chromogenic medium in the bag compartments resulted in about 9\% false positives and 26\% false negatives. The sensitivity of the test, which refers to how well it correctly identifies true \textit{E. coli} positives, was 0.908. The positive predictive value (PPV), which is a measure of the proportion of color positive compartments that are correctly identified to contain \textit{E. coli} based on colony isolation on an \textit{E. coli} chromogenic medium, is also a measure of the precision of the test, which was 0.912. Therefore, based on presumptive \textit{E. coli} colony isolation by streak-plating from color positive CBT bag compartments, the sensitivity and the PPV are both about 91\%. Specificity, which refers to how well the test correctly identifies true negatives, was about 0.753. The negative predictive value (NPV), which is a measure of the proportion of color negative compartments that are correctly identified as negative was about 0.743. Therefore, these values were lower than the PPV and suggest that some color negative bag compartments actually contained \textit{E. coli} based on presumptive colony isolation on \textit{E. coli} chromogenic agar medium. The overall accuracy of the CBT based on presumptive \textit{E. coli} colony isolation from color positive and color negative bag compartments was calculated to be 87\%.
Table 3.6 Confirmation of *E. coli* presence or absence in color positive and color negative CBT chambers based on streak plate *E. coli* colony isolation from chambers on a chromogenic *E. coli* / coliform medium

<table>
<thead>
<tr>
<th>CBT chamber results for <em>E. coli</em></th>
<th>Rapid'E. coli' 2 streak plate outcomes for <em>E. coli</em> detection</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>375</td>
<td>36</td>
</tr>
<tr>
<td>-</td>
<td>38</td>
<td>110</td>
</tr>
<tr>
<td>Total</td>
<td>413</td>
<td>146</td>
</tr>
</tbody>
</table>

Sensitivity = 0.908  
Positive predictive value = 0.912  
Accuracy = 0.870  
Specificity = 0.753  
Negative predictive value = 0.743

*Evaluation of presumptive E. coli positive and false negative isolates with biochemical analysis by EnteroPluri testing*

For the evaluation of biochemical analysis with EnteroPluri testing, to determine bacteria speciation and in particular the identification of *E. coli*, a total of 300 isolates were obtained from presumptive *E. coli* positive sample volumes of CBT color positive chambers, and a total of 38 isolates were obtained from presumptive negative sample volumes that were isolated from CBT color negative chambers when using the Hi-*E. coli* medium. These presumptive positive and negative bacteria isolates were grown from the 559 total archived and frozen CBT isolates from bag chambers. Therefore, 73% were tested from stored and frozen streak plate positive presumptive *E. coli* isolates from color positive chambers and 26% were tested from stored and frozen streak plate presumptive negative isolates from color negative bag chambers. These
isolates were further streaked onto non-selective tryptic soy agar plates and a single colony from each streaked plate was picked and analyzed with the EnteroPluri test. The results of these biochemical analyses are recorded in Table 3.7 below.

According to the results of the EnteroPluri tests, the CBT results in about 3% false positives and 3% false negatives. Out of false positives and true negatives, 11% are identified as *Enterobacter cloacae* and 90% are identified as *Klebsiella pneumonia*, both of which are coliform bacteria of possible fecal origin. For false positives (n=9), about 56% of the isolates are identified as *E. cloacae* (n=5), and 44% of the isolates are identified as *K. pneumonia* (n=4). For true negatives (n=37), 100% of the isolates were identified as *K. pneumonia* (n=37). The positive predictive value (PPV) from the EnteroPluri results is 97% and the negative predictive value (NPV) is 97.4%. The specificity is 80.4% and the sensitivity is 99.7%. The overall accuracy of the CBT was calculated to be 97%.

### Table 3.7 Biochemical speciation using the EnteroPluri system for bacteria isolates from CBT *E. coli* test chambers based on color positivity or negativity in CBT bag chambers

<table>
<thead>
<tr>
<th>Enteropluri biochemical outcomes for <em>E. coli</em> on streak plate isolates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBT bag chamber positivity or negativity for <em>E. coli</em></td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>292</td>
</tr>
<tr>
<td>Sensitivity = 0.997</td>
<td>Positive predictive value = 0.970</td>
</tr>
<tr>
<td>Specificity = 0.804</td>
<td>Negative predictive value = 0.974</td>
</tr>
</tbody>
</table>

| CBT bag chamber positivity or negativity for *E. coli* | + | - |
|---|---|
| Total | 291 | 9 | 300 |
| Sensitivity = 0.997 | Positive predictive value = 0.970 |
| Specificity = 0.804 | Negative predictive value = 0.974 |

Sensitivity = 0.997  
Positive predictive value = 0.970  
Accuracy = 0.970  
Specificity = 0.804  
Negative predictive value = 0.974
Evaluation of presumptive *E. coli* positive and false negative isolates from color positive and color negative bag chamber by molecular analysis using qPCR for the *uidA* gene target

Molecular analysis by qPCR for the *uidA* gene target was performed on a total of 240 presumptive *E. coli* positive isolates obtained from CBT color positive chambers, and a total of 28 presumptive *E. coli* negative isolates obtained from CBT color negative chambers when using the Hi-*E. coli* medium in the CBT. These presumptive *E. coli* positive and negative sample isolates were grown from the 559 total archived and frozen CBT isolates from bag chambers. Of these colony isolates tested 58% were stored and frozen streak plate color positive isolates and 19% tested were from stored and frozen streak plate color negative isolates. These isolates were regrown and a crude DNA extraction was performed. The extraction was then analyzed by qPCR for the *uidA* gene. The results of these analyses are recorded in Table 3.8 below.

According to the results of qPCR for the presence of the *uidA* gene, the CBT results in about 5% false positives of color positive bag chambers that are negative by qPCR for the *uidA* gene and 0% false negatives of color negative bag chambers that are negative by qPCR for the *uidA* gene. The positive predictive value (PPV) from the qPCR results is 95.4% and the negative predictive value (NPV) is 100%. The specificity is 71.8% and the sensitivity is 100%. The overall accuracy of the CBT was calculated to be 95.9%.
Table 3.8 Molecular speciation of *E. coli* by qPCR for the uidA gene of bacteria isolates from CBT *E. coli* test chambers based on color positivity or negativity in bag chambers

<table>
<thead>
<tr>
<th>CBT bag chamber positivity or negativity for <em>E. coli</em></th>
<th>uidA gene qPCR outcomes on streak plate isolates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>229</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>28</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>268</strong></td>
</tr>
<tr>
<td><strong>Sensitivity = 1</strong></td>
<td>Positive predictive value = 0.954</td>
<td></td>
</tr>
<tr>
<td><strong>Specificity = 0.718</strong></td>
<td>Negative predictive value = 1</td>
<td></td>
</tr>
</tbody>
</table>

*Evaluation of presumptive *E. coli* positive and false negative isolates with MALDI-TOF MS molecular analysis*

Evaluation of molecular analysis by MALDI-TOF MS was performed on a total of 323 presumptive *E. coli* positive isolates from CBT color positive chambers, and a total of 38 presumptive negative isolates from CBT color negative chambers when using the Hi-*E. coli* medium. These presumptive *E. coli* positive isolates and false negative isolates were grown from the 559 total archived and frozen CBT colony isolates. Therefore, 79% of the tested isolates were from stored and frozen streak plate color positive samples and 26% of the tested isolates from stored and frozen streak plate color negative samples. These colony isolates were streaked on non-selective tryptic soy agar plates and a single colony was picked and analyzed by MALDI-TOF MS. The results of these analyses are recorded in Table 3.9 below.
According to the results of the MALDI-TOF MS, the CBT results in about 11% false positives for *E. coli* and 0% false negatives for *E. coli*. Of the color positive presumptive *E. coli* that were false positives based on MALDI-TOF MS analysis, a large majority, 75% are identified as *K. pneumonia*. A pie chart graph of the bacteria speciation of *E. coli* false positive isolates from color positive bag chambers as identified by MALDI-TOF MS is shown below in Figure 3.3. The positive predictive value (PPV) from the MALDI-TOF MS results is 89.2% and the negative predictive value (NPV) is 100%. The specificity is 52.1% and the sensitivity is 100%. The overall accuracy of the CBT was calculated to be 90.3%.

**Table 3.9 Molecular speciation of *E. coli* by MALDI-TOF MS of bacteria isolates from CBT *E. coli* test chambers based on color positivity or negativity in bag chambers**

<table>
<thead>
<tr>
<th>CBT bag chamber positivity or negativity for <em>E. coli</em></th>
<th>MALDI-TOF MS outcomes for <em>E. coli</em> on streak plate isolates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CBT bag chamber positivity or negativity for <em>E. coli</em></td>
<td>228</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>Total</td>
<td>288</td>
<td>73</td>
</tr>
</tbody>
</table>

Sensitivity = 1
Positive predictive value = 0.892
Accuracy = 0.903

Specificity = 0.521
Negative predictive value = 1
Summary of CBT performance outcomes based on identification of bacteria isolates from bag compartments by confirmatory tests for identification of presumptive E. coli

The CBT performance outcomes described above are summarized in Table 3.10. Only a percentage of presumptive positive and negative E. coli isolates were tested biochemically with EnteroPluri, by qPCR for the uidA gene, and by MALDI-TOF MS for molecular identification. The reason for characterization of only a fraction of total presumptive E. coli isolates obtained is because some samples were not recoverable by culture methods after being archived in the freezer. However, based on testing a representative number of presumptive E. coli isolates by the different confirmatory test methods, CBT sensitivity was found to range from 91-100%; the specificity ranged from 52-80%; the positive predictive value ranged from 89-95%; the negative predictive value ranged from 74-100%; and the accuracy ranged from 87-97%. The false positive presumptive E. coli isolates were found to be mostly thermotolerant coliforms like Klebsiella

---

2 False positive here refers to (color positive CBT bag chambers, color positive streak-plate results, and non-E. coli MALDI-TOF MS results)
spp., *Enterobacter* spp, and *Citrobacter* spp. by EnteroPluri biochemical tests and MALDI-TOF MS molecular analysis, about 89% and 75% of the presumptive *E. coli* false positives, respectively, were found to be *K. pneumonia*, which is a thermotolerant coliform. In addition, 84% of the total presumptive *E. coli* false positives identified via MALDI-TOF MS were found to be thermotolerant coliforms that are closely related to *E. coli* and of potential fecal origin.

### Table 3.10 Summary results of different methods to analyze CBT performance based on confirmation of presumptive *E. coli* positivity or negativity

<table>
<thead>
<tr>
<th>Method</th>
<th># (%) of + isolates tested</th>
<th># (%) of - isolates tested</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streak Plate colonies</td>
<td>411 (100)</td>
<td>148 (100)</td>
<td>0.908</td>
<td>0.753</td>
<td>0.912</td>
<td>0.743</td>
<td>0.87</td>
</tr>
<tr>
<td>EnteroPluri biochemical</td>
<td>300 (73)</td>
<td>38 (26)</td>
<td>0.997</td>
<td>0.804</td>
<td>0.907</td>
<td>0.974</td>
<td>0.97</td>
</tr>
<tr>
<td>qPCR for uidA</td>
<td>240 (58)</td>
<td>28 (19)</td>
<td>1</td>
<td>0.718</td>
<td>0.954</td>
<td>1</td>
<td>0.959</td>
</tr>
<tr>
<td>MALDI-TOF MS</td>
<td>323 (79)</td>
<td>38 (26)</td>
<td>1</td>
<td>0.521</td>
<td>0.892</td>
<td>1</td>
<td>0.903</td>
</tr>
</tbody>
</table>

PPV = positive predictive value; NPV = negative predictive value

### 3.4 Discussion

Based on the Pearson’s Chi-Square results, it can be assumed that there is an association between the *E. coli* MPN results of the Hi-*E. coli* medium in the CBT and Colilert medium in the CBT across all temperatures evaluated (i.e. 27°C, 37°C or 44.5°C), when categorized as presence/absence and when categorized into WHO risk strata categories. These results are also strongly and positively correlated when categorized by presence/absence and WHO risk strata across all incubation temperatures evaluated based on the Pearson’s R. Performance of the CBT for *E. coli* analysis using the newly developed chromogenic Hi-*E. coli* medium compared to
fluorogenic Colilert, as the reference standard medium, by ANOVA regression with log10(MPN) concentration as the dependent variable resulted in no significant difference in performance, with a regression coefficient of -0.09 and a p-value of 0.18. Likewise by logistic regression comparing MPN < 48.3 vs >=48.3 as the dependent variable between Hi-\textit{E. coli} medium compared to fluorogenic Colilert medium also resulted in no significant difference in performance, with regression coefficient of -0.17 and a p-value of 0.45. The regression analyses also found no effect of test culture medium type on the odds of reporting MPN \textit{E. coli} concentrations greater than or equal to 100 per 100 mL or 1.5 per 100 mL. These regression results for \textit{E. coli} concentrations detected by the CBT method between the two types of culture media suggest that CBT with chromogenic Hi-\textit{E. coli} medium versus with Colilert fluorogenic medium does not significantly change estimates of MPN concentrations of \textit{E. coli} per 100 mL.

Water samples with color positive compartments when incubated at higher temperatures will likely be identified as \textit{E. coli} in confirmatory tests. This color change is a result of sufficient cleavage of the chromogenic substrate by the bacteria to produce the distinctive visible color change. When comparing the CBT with Hi-\textit{E. coli} medium and Colilert medium at temperatures of 27°C and 37°C, the results of regression analysis indicate no effect of the temperature used on log10(MPN) concentration nor on the odds of reporting MPN greater than or equal to 100, 48.3, or 1.5. Incubating a sample at 44° C versus 27° C indicates a statistically significant difference in log10(MPN) at the 5% level, however the increase in the arithmetic MPN value at 44 °C is only 1.22 MPN per 100 mL. Based on the results of regression analysis, the odds of having an MPN greater than or equal to 48.3/100 mL when the temperature is 44° F are 1.63 times larger than the odds when the temperature is 27° C. However that increase was not found to be significant at the 5% level. Therefore, there is not a statistically significant difference between MPN/100 mL.
results when incubating at 44° C versus 27° C and the arithmetic MPN changes are slight, less than 2 MPN/100 mL in concentration difference.

The majority of bacteria speciated from color change presumptive positive bag compartments of the CBT were identified as *E. coli* via both molecular and biochemical methods, with a false positive rate of 3-11%. The false negative rates were found to be from 0-26%, depending on the confirmatory analytical method. The false positive isolates that were color change positive but did not speciate as *E. coli* were found to be other thermotolerant coliform species, such as *Klebsiella* spp., *Enterobacter* spp, and *Citrobacter* spp. For example, the EnteroPluri biochemical speciation results and MALDI-TOF MS speciation results found about 89% and 75% of false positives, respectively, to be *K. pneumonia*, a thermotolerant coliform that can be associated with fecally contaminated waters. In addition 84% of the false positive non-*E. coli* species identified via MALDI-TOF MS also were found to be various thermotolerant coliform species. These false positive identifications of non-*E. coli* coliforms, in combination with the low false negative rate, demonstrate that the CBT provides a conservative estimate of *E. coli* by marginally overestimating the presence of fecal bacteria detection in water to protect human health. The accuracy of the CBT was calculated to be 87-97% via the molecular and biochemical methods utilized. This result for accuracy of the CBT is consistent with the findings of Stauber et al who reported the test accuracy to be 95% (Stauber et al., 2014).

There are a number of limitations to this study. One limitation is that different concentrations of X-gluc for the culture medium were not tested on field samples of water. The selection of the X-gluc concentration in the medium was made from results for positive control strains of *E. coli* tested in spiked reagent water samples from previous laboratory experiments done by the manufacturer. Another limitation is that only surface waters from a local creek were
sampled and analyzed in this study, which may limit the representativeness of the results, perhaps due to the limited variability of the microbial community in the sample water. Also, no pure cultures of known bacteria strains were spiked into natural water at known concentrations to determine the recovery and detection efficiency. Yet another limitation of the study was the loss of viability of some frozen isolates for later identification and the resulting sample size differences of presumptive positives and negatives, which were not constant among the various experiments. In addition, no CBT analysis was conducted on the version of the CBT to detect and quantify H$_2$S producing bacteria. Previous studies demonstrate that the H$_2$S medium is cheaper and that H$_2$S producing bacteria are comparable fecal indicator bacteria to *E. coli* for detecting fecal contamination (McMahan et al., 2011).

### 3.5 Conclusions

The performance of the CBT test using chromogenic Hi-*E. coli* medium versus using fluorogenic Colilert medium was not significantly different for detecting positive results and quantifying MPN concentration in natural surface water containing *E. coli*. There were no statistically significant differences in *E. coli* MPN concentration results found when incubating at 44° C versus 27° C. The differences in MPN concentrations were slight, averaging less than 2 MPN/100 mL difference in concentration. Therefore incubation temperatures of 27°C, 37°C, and 44°C should not give significant differences in CBT MPN concentrations for *E. coli* in water. The majority of presumptive positive *E. coli* based on the distinctive color change in the water of bag compartments were subsequently confirmed to be *E. coli* by biochemical and molecular methods. Of the non-*E. coli* false positive isolates speciated, 75-88% of them speciated biochemically via Enteropluri and molecularly by MALDI-TOF MS were found to be
thermotolerant coliform species like *Klebsiella, Enterobacter,* and *Citrobacter.* These false positive species identified are considered potential indicators of fecal contamination. The CBT is documented to provide a reliable estimate of *E. coli* and a somewhat more conservative estimate of fecal contamination concentrations in water. Overall, the results from this study calculated the accuracy of the CBT to be 87-97%. Therefore, it is concluded that the CBT can be used to identify and quantify *E. coli* with a degree of accuracy comparable to standard *E. coli* media such as Colilert across incubation temperatures of 27°C, 37°C, and 44°C. The CBT appears to have the potential to successfully overcome many of the existing barriers to microbial water quality testing in low resource settings.
4.1 Introduction

An estimated 748 million people worldwide lack access to safe drinking water sources, putting them at risk for water-borne illnesses (WHO/UNICEF, 2014). Regulated drinking water supplies are typically monitored for water quality, including the presence of fecal bacteria, but only at the water source, after water treatment, and in the water distribution system under the jurisdiction of the water supplier (WHO, 2004). Microbial water quality monitoring of regulated water supplies in many developing countries is often infrequent (Ashbolt, 2004; Tallon et al., 2005; Riesbro et al., 2012). In non-regulated water supplies (private wells, rainwater harvesting cisterns or private household and community water supplies exempt from government regulation or oversight) water is virtually never tested for quality (Riesbro et al., 2012). Therefore, from local to global scale, the quality and safety of household drinking water is rarely if ever measured and essentially unknown.

Although the Millennium Development Goals (MDG) of the UN have set a target to reduce by half the number of households lacking sustained access to safe water (and sanitation) by the year 2015, there are limited microbial water quality data for improved or unimproved water sources or for the on the microbial safety of household drinking water quality (Onda et al., 2012; Bain et al., 2014). The Joint Monitoring Programme (JMP) of the UN has no reliable basis to track progress towards the water target due to the unavailability of simple and affordable methods to test directly the microbial quality of household drinking water (Bain et al., 2012b).
Instead, the JMP uses household surveys to determine whether the source of drinking water supply is either improved or unimproved. According to this simple classification system, global access to improved water sources increased between 1990 and 2015, and Target 7c was considered to be “on track” and actually met in 2010, five years ahead of schedule (WHO/UNICEF, 2014).

However, evidence from UNICEF surveys indicated in multiple countries one half of protected dug wells and one third of protected springs and boreholes were microbially contaminated (WHO/UNICEF, 2010). Other evidence, including the rapid assessment of drinking-water quality projects (RADWQ) and global modeling projections based on RADWQ data, has also called into question the microbiological safety of many water sources classified as improved under the definitions used by the JMP (Onda et al., 2012). Furthermore, there continue to be regional disparities in safe water access (WHO/UNICEF, 2010; WHO/UNICEF, 2014). Microbial water quality is known to differ substantially between rural and urban areas, and the majority of users of unimproved sources live in Africa and Southeast Asia (Bain et al., 2014). To decrease the lack of access to safe drinking water and disparities in access, it is necessary to identify microbially unsafe drinking waters in order to take actions improve them. Hence, simple, portable, and accessible tests to quantify the microbial quality of household drinking water in the field are much needed. The Compartment Bag Test was created to meet this need.

A portable and simple test for determining the microbiological safety of household drinking water has been developed by Dr. Mark D. Sobsey and Dr. Lanakila McMahan of the University of North Carolina Gillings School of Global Public Health (McMahan et al., 2009; McMahan et al., 2011; Stauber et al., 2014). The Compartment Bag Test (CBT) detects and quantifies target fecal indicator, such as *Escherichia coli* or H$_2$S producing bacteria in 100 mL.
volumes of water as a Most Probable Number (MPN) estimate. The CBT is a clear, sterile, polyethylene bag (Nasco) that was modified to provide internal compartments of 56, 30, 10, 3, and 1mL volumes, totaling 100mL. Using the CBT, the presence and concentrations of target fecal bacteria in water, food, and other environmental media can be determined, thereby making it possible to evaluate safe water access, identify unsafe water, and reliably track progress towards the water target of the MDGs and the water target of the post-2015 Sustainable Development Goals. Such microbial data on water quality makes it possible to take actions to improve unsafe water quality and thereby reduce enteric infectious disease risks from waterborne exposure sources.

The purpose of this study was to evaluate the performance of the CBT in quantifying microbial safety of household drinking water when performed by DHS field survey staff in Peru and to document the potential for its use in microbial water quality monitoring within DHS field data collection. The study was done by DHS survey teams for three geographically distinct and representative regions of Peru. A technician trained to perform the CBT on drinking water samples collected from households was added to each of the three survey teams. The results for *E. coli* concentration in 100 mL samples of household drinking water from the CBT performed by survey staff in the field were compared statistically to the results from parallel analysis of the same water samples by trained analysts in reference laboratories using both the CBT and a standard membrane filtration (MF) method.

### 4.2 Methods

Before implementing the field study, formal training on the use of the CBT method was provided in Lima in the government building of the Instituto Nacional de Estadística e
Informática (INEI). Field technicians for all three field survey regions and the laboratory technician for the Lima reference laboratory were in attendance. The process of how to collect, label and process samples, use an incubator with either electricity or an alcohol lamp to heat it, read, and record CBT results, maintain cold and sample transport chain were fully introduced and explained. Each technician in attendance collected and processed a water sample and results were read and recorded during the training. An informal training, similar to the formal training but less extensive, was provided on site for laboratory technicians in the reference laboratories of Loreto and Junín. All technicians received CBT training materials for reference and use when needed.

During March-June 2011, 704 households were surveyed in three regions in Peru by trained field teams from INEI: Lima (Pacific coast), Junín (Andes mountains), and Loreto (Amazon jungle). Consistent with the sampling methodology of the DHS, INEI randomly selected households to participate in the survey and provide a sample of household drinking water (Macro, 1996). Consenting households provided three 100mL drinking water samples collected by the method that would have been used to get a volume to drink and then transferring the water to sample collection bottles. Water samples analyzed for microbial water quality were collected in bottles containing sodium thiosulfate to neutralize any chlorine. The water samples were identified and tracked using a unique sample code that “masked” the identification of the households. Collected water samples were transported and stored at or below 10°C with ice packs until analysis.

A member of the DHS survey team using a portable free chlorine test kit with N,N Diethyl-1,4 Phenylenediamine Sulfate (DPD) (Hach) to measure free chlorine residual of collected tap water as described in the DHS guidelines; and with the CBT was used to analyze
one of the 100 mL household drinking water samples for *E. coli* in the field. Two replicate 100 mL samples were transported to the reference laboratory and analyzed with the CBT and MF. Membrane filters were incubated overnight for 20-28 hours at 37°C on absorbent pads with the same *E. coli* liquid culture medium as used with the CBT (HiMedia Laboratories, Mumbai, India). *E. coli* colonies were detected by their distinctive blue color. In instances where electricity was not available in the field, the samples were incubated at room temperature (26-33°C) for at least 30 hours, or incubated in a modified electric incubator (ThinkGeek™) fitted with vent holes and heated by an alcohol burner to achieve a temperature of 36-38°C (Brown et al., 2011).

Positive results for *E. coli* in the CBT were indicated by a distinctive blue color change within bag compartments. This color change resulted from the hydrolysis activity of the β-glucuronidase enzyme unique to *E. coli* acting on the chromogenic Beta-D-glucuronide substrate, Indoxyl-Beta-D-glucuronide (X-Gluc), in the medium (Watkins et al., 1988). The concentration of *E. coli* in each compartment bag was calculated using the U.S. EPA MPN calculator, which uses the volumes of the positive and negative compartments to estimate a MPN/100mL (Cochran, 1950; Klee, 1993).

Water quality data were transferred to Microsoft Excel for analysis. All data were entered twice to minimize data entry error. The data were then subjected to statistical analysis (GraphPad Prism 5, GraphPad Software, Inc, 2009 & Stata 10.1, StataCorp, College Station, Texas, USA) to determine the extent of agreement in measured concentrations of *E. coli* in household water samples as measured by: (1) the DHS survey teams using the CBT, (2) the reference laboratories using the CBT, and (3) the reference laboratories using a standard membrane filtration test. Data analysis employed non-parametric matched pairs analyses and ANOVAs to determine the extent
of agreement of the three different *E. coli* measurements of the same water sample. A paired t-test or a non-parametric equivalent was used to determine if the CBT test done by the survey team and done by the reference laboratory on the same water samples gave equivalent results. The *a priori* threshold for statistical significance was $\alpha=0.05$.

4.3 Results

*Comparison of field and laboratory sample holding times and incubation temperatures*

Most water samples were analyzed for *E. coli* within 24 hours of sample collection. Samples for which holding times were excessive by being >48 hours, of which there were 39 (5% of the total samples), were excluded from all statistical analyses (USEPA, 2008). The holding time in the Amazon jungle region for laboratory analysis was especially lengthy, since many Amazon jungle towns are only accessible by boat. See Table 4.1 below for the average water sample holding times for the field and laboratory samples across the three field site locations.

<table>
<thead>
<tr>
<th>Table 4.1 Average water sample holding time for field and laboratory analysis at all three field sites in Peru, 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Average Holding Time</strong></td>
</tr>
<tr>
<td>Field Analysis</td>
</tr>
<tr>
<td>Laboratory Analysis</td>
</tr>
</tbody>
</table>

The incubation method and thus incubation temperatures varied depending on the region and availability of electricity. In the Amazon jungle region, because electricity was unreliable and the ambient temperature was always above 25°C during the survey period, only ambient temperature incubation was utilized. Table 4.2 indicates the number of samples incubated by
different incubation methods and average temperatures at each field site and average temperatures for incubation at all three field sites.

Table 4.2 CBT incubation methods and temperatures used at all three field sites

<table>
<thead>
<tr>
<th>Method of Incubation (Average temperature)</th>
<th>Lima 20°C-25°C</th>
<th>Loreto 27°C-30°C</th>
<th>Junín 18°C-22°C</th>
</tr>
</thead>
<tbody>
<tr>
<td># Incubator (37°C)</td>
<td>252</td>
<td>0</td>
<td>178</td>
</tr>
<tr>
<td># Modified Incubator (30°C-45°C)*</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td># Ambient incubation (26-33°C)</td>
<td>0</td>
<td>232</td>
<td>0</td>
</tr>
</tbody>
</table>

*Modified incubation used the alcohol burner heat source in the vented electric incubator.

Comparison of field and laboratory results of E. coli concentration in household drinking water samples

The E. coli concentrations as medians, arithmetic means, and 95% confidence limits (CL) and intervals from the results of the CBT as MPN/100 mL and MF as colony forming units, (CFU)/100 mL, are shown in Table 4.3 for the water samples of all regions combined. Given that only households from 3 departments, out of 24, were sampled, it is important to note that these data are not necessarily representative of Peru as a whole. Table 4.3 represents only the 665 samples that were processed within 48 hours of sample collection. Across all three survey regions, the average E. coli concentrations found by field CBT, laboratory CBT and laboratory MF was 16.8-17.5 E. coli/100mL and have overlapping 95% confidence limits shown in Figure 4.1. The highest concentrations of E. coli in household drinking water samples were found in Loreto (Amazon jungle) at >101CFU/100mL, and the lowest in Lima (Pacific coast) at <CFU/100mL. Within each region, E. coli concentrations measured in the field and laboratory by
the CBT and measured in the laboratory by MF as arithmetic means were comparable and have overlapping 95% confidence limits.

Table 4.3 Overall summary of median, arithmetic mean, and lower and upper 95% confidence limits (CL) of *E. coli* concentrations from each test of household drinking water samples

<table>
<thead>
<tr>
<th></th>
<th>Field CBT (<em>E. coli</em> in MPN/100 mL)</th>
<th>Laboratory Membrane Filtration (<em>E. coli</em>/100 mL)</th>
<th>Laboratory CBT (<em>E. coli</em> in MPN/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Households</td>
<td>665</td>
<td>665</td>
<td>665</td>
</tr>
<tr>
<td>Mean</td>
<td>17.5</td>
<td>17.2</td>
<td>16.8</td>
</tr>
<tr>
<td>Median</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Maximum</td>
<td>101.0</td>
<td>101.0</td>
<td>101.0</td>
</tr>
<tr>
<td>Lower 95% CL</td>
<td>14.8</td>
<td>14.7</td>
<td>14.3</td>
</tr>
<tr>
<td>Upper 95% CL</td>
<td>20.1</td>
<td>19.8</td>
<td>19.4</td>
</tr>
</tbody>
</table>

Figure 4.1 Arithmetic means and 95% confidence intervals for *E. coli* concentrations in household drinking water samples by each test, and by each survey region – Lima (n=252), Loreto (n=181), Junin (n=232)
The statistical comparison for all three survey locations combined and separated by site are shown in Table 4.4. Because the data on *E. coli* concentrations did not conform to a normal probability distribution, nonparametric statistical methods were used. For the 665 household water samples of all regions combined, there were no statistically significant differences in results for measured concentrations of *E. coli* between the reference laboratory (MF and CBT) and field analysis (CBT) by Repeated Measures ANOVA (p=0.77) or by Friedman Test (Nonparametric Repeated Measures ANOVA, p=0.25). Likewise, Wilcoxon matched-pairs signed-ranks tests showed that there were no statistically significant differences in measured *E. coli* concentrations of the same household water samples of all survey locations combined between the laboratory or field CBT results (p=0.50, Spearman’s r=0.88), between the laboratory CBT and laboratory MF results (p=0.43, Spearman’s r=0.88), or between the field CBT and laboratory MF results (p=0.84, Spearman’s r=0.76).

**Table 4.4 Summary of matched pair non-parametric test results and Spearman’s rank correlations for *E. coli* concentrations in household water samples of all three survey locations combined and separate**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>p-value for either Friedman’s Test for Non-Parametric Repeated Measures ANOVA(^a) or for Wilcoxon matched-pairs signed-ranks tests(^b)</th>
<th>Spearman’s Rank Correlation(R)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All Locations</strong></td>
<td>0.25(^a)</td>
<td>0.88</td>
</tr>
<tr>
<td>Field CBT vs. Laboratory CBT</td>
<td>0.50(^b)</td>
<td>0.80</td>
</tr>
<tr>
<td>Field CBT vs. Laboratory MF</td>
<td>0.84(^b)</td>
<td>0.76</td>
</tr>
<tr>
<td>Laboratory CBT vs. Laboratory MF</td>
<td>0.43(^b)</td>
<td>0.88</td>
</tr>
<tr>
<td><strong>Lima (Pacific coast)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field CBT vs. Laboratory CBT</td>
<td>0.68(^b)</td>
<td>0.81</td>
</tr>
<tr>
<td>Field CBT vs. Laboratory MF</td>
<td>0.07(^b)</td>
<td>0.60</td>
</tr>
<tr>
<td>Laboratory CBT vs. Laboratory MF</td>
<td>0.14(^b)</td>
<td>0.62</td>
</tr>
<tr>
<td><strong>Loreto (Amazon jungle)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Field CBT vs. Laboratory CBT</td>
<td>0.29(^b)</td>
<td>0.90</td>
</tr>
</tbody>
</table>
Field CBT vs. Laboratory MF 0.37\(^b\) 0.88  
Laboratory CBT vs. Laboratory MF 0.03\(^b\) 0.95  
**Junín (Andes mountains)**  
Field CBT vs. Laboratory CBT 0.76\(^b\) 0.61  
Field CBT vs. Laboratory MF 0.16\(^b\) 0.60  
Laboratory CBT vs. Laboratory MF 0.08\(^b\) 0.88  
*Bold values indicate significant pairings*

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

*E. coli* occurrence and concentrations in relation to residual chlorine

The differences in the percentage of household water samples that were positive for *E. coli* categorized according to their chlorine concentrations were statistically significant by Pearson Chi-Square test (p = 0.001) and are shown in Table 4.5. Households with > 0.5 mg/L free chlorine in sampled water had the highest percentage of samples negative for *E. coli* at 84%. However, 16% of these household water samples (n=19) with >0.5 mg/L free chlorine were positive for *E. coli*. When all samples were included in the analysis (including those with *E. coli* non-detects assigned an *E. coli* concentration of 0.1 MPN/100 mL), arithmetic and geometric mean *E. coli* concentrations were lowest in household water with >0.5 mg/liter free chlorine (4.4MPN/100mL and 0.19MPN/100mL, respectively), and then increased as chlorine
concentration decreased. Based on a Kruskal Wallis test, the median concentrations of *E. coli* for each category of chlorine concentration in household water samples was found to be significantly different (*p* = 0.0003).

**Table 4.5** *E. coli* occurrence and concentrations for household drinking water with different concentrations of free chlorine

<table>
<thead>
<tr>
<th>Chlorine Concentration (mg/liter)</th>
<th>Number of Samples*</th>
<th>Percentage of Samples negative for <em>E. coli</em></th>
<th><em>E. coli</em> Arithmetic Mean (MPN/100mL) (95% CLs)</th>
<th><em>E. coli</em> Geometric Mean (MPN/100mL) (95% CLs)*</th>
<th><em>E. coli</em>/100mL Minimum/Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;=0.5</td>
<td>120</td>
<td>84%</td>
<td>4.4 (1.0-7.8)</td>
<td>0.19 (0.14-0.26)</td>
<td>(0,101)</td>
</tr>
<tr>
<td>0.1 to &lt; 0.5</td>
<td>98</td>
<td>76%</td>
<td>15.3 (8.2-22.4)</td>
<td>0.41 (0.25,0.68)</td>
<td>(0,101)</td>
</tr>
<tr>
<td>&lt;0.1</td>
<td>484</td>
<td>67%</td>
<td>18.7 (15.5-22.0)</td>
<td>0.62 (0.49,0.80)</td>
<td>(0,101)</td>
</tr>
</tbody>
</table>

* 59 samples did not have chlorine residual concentrations because the water was taken from a non-treated source, was bottled water or the test could not be performed. A MPN concentration of 0.1/100mL was assigned to all samples in which no *E. coli* were detected (recorded initially as <1 MPN/100mL) in order to be able to calculate geometric mean concentrations.

*E. coli* concentrations according to water source and treatment

Water sources classified as improved or unimproved were examined for *E. coli* occurrence and decimal concentration categories defined by the World Health Organization, based on CBT field results and MF laboratory results and shown in Tables 4.6 and 4.7.

**Table 4.6** Number and percentage of field CBT analyzed household drinking water samples in each WHO decimal category of *E. coli* concentration for improved and unimproved sources

<table>
<thead>
<tr>
<th>Field CBT Results, <em>E. coli</em>/100mL</th>
<th>Number (Percentage) of Households</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Improved</td>
</tr>
<tr>
<td>&lt;1</td>
<td>353 (74)</td>
</tr>
<tr>
<td>1-9</td>
<td>43 (9)</td>
</tr>
<tr>
<td>10-99</td>
<td>27 (5)</td>
</tr>
<tr>
<td>&gt;100</td>
<td>58 (12)</td>
</tr>
<tr>
<td>Total</td>
<td>481 (100)</td>
</tr>
</tbody>
</table>
Table 4.7 Number and percentage of laboratory MF analyzed household drinking water samples in each WHO decimal category of *E. coli* concentration for improved and unimproved sources

<table>
<thead>
<tr>
<th>Laboratory MF Results, <em>E. coli</em>/100mL</th>
<th>Number (Percentage) of Households</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Improved</td>
</tr>
<tr>
<td>&lt;1</td>
<td>386 (80)</td>
</tr>
<tr>
<td>1-9</td>
<td>35 (7)</td>
</tr>
<tr>
<td>10-99</td>
<td>57 (12)</td>
</tr>
<tr>
<td>&gt;100</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Total</td>
<td>481 (100)</td>
</tr>
</tbody>
</table>

The number of samples in the different *E. coli* concentration categories depending on whether measured in the field by CBT or laboratory by MF categories was compared with Wilcoxon matched-pairs signed-ranks tests. In the category of improved water sources, the number of samples in the different *E. coli* concentration categories between field CBT results and laboratory MF results were not significantly different (*p* = 0.969). Likewise, in the category of unimproved water sources, the number of samples in the different *E. coli* concentration categories between field CBT results and laboratory MF results were also not significantly different (*p* = 0.504). These results further document that field CBT results are equivalent to laboratory MF results not only when comparing *E. coli* detection and concentrations, but also when regarding information about the microbial safety of drinking water based on the WHO drinking water standards (WHO, 2011). The CBT results by reference laboratories are reliably predicted by field analyzed CBT results and also equivalent to that of a standard method done in a laboratory.

4.4 Discussion

Based on the current classification system that identified drinking water as either improved or unimproved, available laboratory data suggests that the MDG target to improve
access to “safe” water has already been achieved (WHO/UNICEF, 2012; 2014). However, analysis employing statistical models using applied microbiological water quality results from the Rapid Assessment of Drinking Water Quality (RADWQ) studies across five countries demonstrate a shortfall (10%) of the global population towards the water target in 2010 (Onda et al., 2012). Furthermore, the results of this study indicate that between 20-26% of household drinking water samples from improved sources contained measurable concentrations of \textit{E. coli} bacteria in 100 mL sample volumes. Therefore, a more objective and rational method based on quantitative microbial water quality data is needed to better characterize water supplies as safe or unsafe in order to better manage drinking water safety. The JMP along with other organizations that evaluate progress towards the water target of the post-2015 Sustainable Development Goals should focus on how to perform systematic microbiological water quality testing of household water samples on a national scale in applicable countries.

In this study, measurable concentrations of \textit{E. coli} bacteria were found in water samples with measurable chlorine residuals. This finding may seem implausible because of the widespread use of chlorine to disinfect drinking water and achieve non-detectable levels of fecal bacteria in 100mL sample volumes. However, \textit{E. coli} have been detected previously in drinking water samples with measurable chlorine residuals. The presence of detectable levels of \textit{E. coli} and other fecal bacteria in drinking water samples with detectable free chlorine residual could be caused by the presence of injured but culturable fecal bacteria in chlorinated waters, which has been previously documented (McFeters and Camper, 1983). Other possible explanations for the presence and persistence of such bacteria in drinking water with measurable chlorine residuals is the presence of fecal bacteria in aggregates or clumps and their association with or presence in other, protective particles of organic matters, such as from fecal matter or in biological particles.
released from biofilms. Another possible explanation for *E. coli* detection in household drinking water samples even when free chlorine was present in household drinking water samples is that these water samples were taken from different water containers or sources used by the household or at different times. For example, 18 of these 19 households said that they stored drinking water. It is possible that the water sample used to measure *E. coli* was taken from the storage container but the sample for free chlorine analysis was perhaps taken directly from the tap, according to the established guidance in the DHS manual (Macro, 1996).

The CBT is a promising tool that provides an opportunity to do widespread and routine microbiological testing of household drinking water on samples collected during household surveys. The CBT is simple, portable, and performs similarly to other more complex, less portable, and more expensive tests that are unsuitable for field use in low-resource settings. Many of these tests must be done in specialized laboratories by skilled technicians, which can add additional costs (McMahan 2009; 2011; Bain et al., 2012a; Stauber et al., 2014). The CBT is relatively inexpensive, about 5-10USD/sample, and its exact cost depends on the bacteriological medium used to detect the target fecal bacteria of interest (Aquagenx, 2012; McMahan 2009; 2011; Bain et al., 2012a). A new medium, Resorufin β-D-Glucuronide, can potentially be utilized as the chromogenic substrate for detecting *E. coli*, and decrease costs even more (Magro et al., 2014). Averaging across the three survey regions for the entire study, the cost associated with conducting the CBT in the field was estimated to be 3287USD, whereas the cost for MF in the laboratory was estimated at 5443USD, which is almost 50% difference in cost. Because of economies of scale, the costs associated with the CBT can potentially decrease, but the costs associated with regional reference laboratories are unlikely to change. The CBT is particularly advantageous because it does not require electricity or supplemental equipment other than an
incubator where ambient temperatures are unsuitable to grow *E. coli* bacteria (Brown et al., 2011).

This study, to our knowledge, is the first time a field-portable, quantitative water microbiology test has been included in a DHS and its performance independently verified by analysis of the same water samples by expert laboratories using both the same field water microbiology test as well as a standard water microbiology test. The agreement between the field and laboratory data on *E. coli* concentrations in household water samples suggests that the CBT for *E. coli* is an effective method to quantify bacteria of fecal origin in household drinking water overall and is especially amenable to field use. Also, the ambient temperature results of the CBT as performed by field survey workers (n=232) were no different than the CBT results performed by the reference laboratories using standard incubation conditions on the same household water samples. This is especially important because in low-resource settings, an incubator and/or electricity to power it is often not available; therefore, the ability of the CBT to produce reliable results at ambient temperatures is desirable.

One of the limitations of the CBT in its applicability to some types of water samples is its upper detection limit of about 100 *E. coli* MPN/100mL of undiluted water. Many ambient environmental waters can have concentrations >100 *E. coli*/100mL, including those approved for primary contact recreation, such as swimming (Brenniman et al., 1981). This concentration of *E. coli* greatly exceeds the recommended *E. coli* level of the WHO Guidelines for Drinking-water Quality which is none detectable/100mL, and drinking water of even with this *E. coli* concentration will not be fully quantified or distinguished by this test (WHO, 2004). However, the CBT provides actionable information for decision-making about the safety of drinking water because it quantifies and distinguishes the *E. coli* concentrations in 100 mL samples of drinking
water corresponding to the WHO decimal categories of potential health risk, which are <1 and deemed safe or very low risk, 1-9 and considered probably safe and low risk, 10-99 and considered possibly safe but of some potential risk and >100, considered unsafe and a risk to drink (WHO, 2004; 2011).

In situations where all compartments turn positive, indicating exceedance of the highest concentration of bacteria detectable by the CBT and a high-risk likelihood of containing 100 or more \( E.\ coli \) 100mL, the possibility that the \( E.\ coli \) concentration is actually much higher than this value should be considered in regards to the human health risks from exposure. For applications of the CBT to waters expected to have higher concentrations of \( E.\ coli \), it is readily possible to compensate for the lower upper detection limit by first appropriately diluting the water sample in \( E.\ coli \)-free dilution water perhaps 10-fold or more, as has been reported previously for the use of the CBT to analyze a range of different ambient water samples in Atlanta, Georgia, USA (Stauber et al., 2014). Additionally to compensate for the lower upper detection limit, a second or third CBT can be appropriately diluted if general water quality of a given region is known.

Another limitation of the CBT is that it has only five compartments. This limited number of sample sub-volumes results in somewhat broader confidence intervals estimates of MPN concentration of bacteria than those of other MPN tests that employ a greater number of discrete sample volumes. However, the upper 95% confidence limit values of MPN values of the CBT are not so large in magnitude relative to the MPN concentration estimates that the water would be classified differently on the basis of the WHO decimal categories of \( E.\ coli \) concentration/100mL.
4.5 Conclusions

This study shows that the CBT to quantify *E. coli* concentration in 100mL volumes in drinking water can be incorporated into a Demographic Health Survey (DHS) to determine the quality of household drinking water and provide results equivalent to those obtained on the sample water samples using a standard *E. coli* quantification test performed by trained analysts in a standard analytical laboratory. Field DHS surveyors who received a two-day training can perform household water quality testing for *E. coli* by the CBT and achieve results similar to well-trained laboratory technicians in standardized laboratory environments. Furthermore, the ability of the CBT to be done successfully under challenging field conditions in diverse geographic regions ranging from Lima (Pacific coast), Loreto (Amazon jungle), and Junín (Andes mountains) regions of Peru highlight the robustness and applicability of the CBT in a wide range of settings. The successful incorporation of the CBT within the Peruvian DHS provides evidence that should encourage its use for analysis of field household and community drinking water samples to support the JMP proposed water quality targets for the post-2015 Sustainable Development Goals (SDGs), which includes measurement of *E. coli* concentrations as a fecal indicator of drinking water. Further field evaluation of the CBT is recommended in order to more fully determine its reliability and field applicability in *E. coli* detection. However, the results of this study demonstrate the ability of the CBT to be reliably and effectively performed in the field by demographic survey staff and give results comparable to a widely used standard *E. coli* test performed by trained and experienced technicians in a typical microbiology laboratory.
CHAPTER 5: EVALUATION OF THE COMPARTMENT BAG TEST FOR *E. coli* IN A LIBERIAN DEMOGRAPHIC AND HEALTH SURVEY

5.1 Introduction

The Millennium Development Goal (MDG) 7c, calls for halving by 2015 the proportion of the population without sustainable access to safe drinking water (WHO/UNICEF, 2010; WHO/UNICEF. 2012). Within the target, safe drinking water is defined as use of an improved water source, meaning that the water source by nature of construction is protected from outside contamination, specifically fecal matter (WHO/UNICEF, 2010; WHO/UNICEF. 2012). The United Nations (UN) also cites water safety as a basic human right, noting that safe water should be free from contaminants that harm human health. Due to the lack of reliable and nationally representative data on water quality, use of water from an improved source is the accepted proxy indicator for water safety. This proxy determinate of safety, however, is limited as improved sources can produce unsafe water and technologies classified as unimproved can provide safe drinking water (WHO/UNICEF, 2010; Onda et al., 2012). A better measure of water safety would be to measure bacterial contamination of water. The most common indicator of microbial water quality is *Escherichia coli* contamination (WHO, 2004).

While water quality data are needed by countries to monitor the safety of drinking water, there are several challenges that have historically prevented incorporation of water testing into household surveys: most current tests to detect and quantify *E. coli* and other fecal bacteria in water are expensive, time-consuming, and require electricity and specialized technical training (Bain et al., 2012a). To address the need for a simple, portable, self-contained, affordable test to
detect and quantify *E. coli* bacteria in water samples, the Compartment Bag Test (CBT) was developed by the Department of Environmental Sciences and Engineering at the University of North Carolina. The CBT uses the MPN methodology to generate an estimate of *E. coli* contamination following the WHO guidelines (McMahan et al., 2009; McMahan et al., 2011). A 100 mL water sample is combined with chromogenic growth medium specific for *E. coli*, poured into a plastic bag containing internal compartments of different volumes, incubated for 24-30 hours at 35-44.5°C, and each compartment is then scored for a color change from yellow to blue or blue green. The color change is indicative of the presence of *E. coli*, and therefore, by observing the combination of compartments that changed color, it is possible to classify a sample as safe, low risk, intermediate risk, or high risk.

The CBT has proven reliable within a laboratory setting and has shown promising field results when piloted in the 2011 Peru Continuous DHS survey (Stauber et al., 2014). Peru, however, is a middle-income country not a low-income country, and carries out a continuous DHS rather than a standard DHS. The purpose of this study was to evaluate the performance of the CBT in quantifying microbial safety of household drinking water when performed by DHS field survey staff, as well as document the potential for microbial water quality monitoring within regular DHS data collection in an extremely low-resource setting. Liberia was selected as the location to carry out the study because it is one of the least developed countries in the world. If the CBT proves to provide reliable and accurate data in a limited resource setting such as Liberia, the test could be used in future national health surveys and other low resource settings as a method to measure the safety of household drinking water.
5.2 Methods

During June through August of 2013, a subset of households from the Liberian Demographic and Health Survey (LDHS) were chosen to be part of this study as determined by the Liberia Institute of Statistics and Geo-Information Services (LISGIS) (Macro International Inc, 1996). A total of 342 households in the clusters of Montserrado, Margibi, and Bomi counties were surveyed this CBT pilot study. Consenting households provided drinking water samples in a 100 mL plastic bottle that was supplied with the CBT kit and a 300 mL Whirl-Pak® bag. To neutralize any chlorine present in the water sample, the collection containers contained sodium thiosulfate. Each container was labeled with a barcode; corresponding barcodes were also placed on the CBT bag, on a Water Sample Transmittal Form, and on the CBT Data Collection. The presence of barcodes on each item allowed the water samples to be tracked and facilitated linking the data collected in the field to be that collected in the lab. Filled containers were placed in a cooler and stored with ice packs until analysis.

A member of the LDHS team tested a 100 mL sample in the field with the CBT; the other 200 mL sample was transported to the National Water Quality Control Laboratory for quality control (QC) testing. At the laboratory, samples were split into two; one sample underwent membrane filtration (MF) testing to obtain a count of \textit{E. coli} colony forming units (CFUs) per 100 mL of water; the other sample was analyzed using the CBT. Membrane filters were incubated on absorbent pads with the same \textit{E. coli} liquid culture medium as used with the CBT, and \textit{E. coli} colonies were detected as blue colonies. Samples were incubated overnight for 20-28 hours at 44°C. In instances where electricity was not available in the field, the samples were incubated in a modified ThinkGeek™ electric incubator heated by butane and fitted with vent holes (39-44°C).
A positive result for the CBT was indicated by a blue color change within a compartment. This color change resulted from the hydrolysis activity of the β-glucuronidase enzyme unique to *E. coli* acting on the chromogenic Beta-D-glucuronide substrate Indoxyl-Beta-D-glucuronide (X-Gluc) in the medium (Watkins et al., 1988). The concentration of *E. coli* in each bag was calculated using the U.S. EPA MPN calculator, which uses the volumes of the positive and negative compartments to determine the MPN/100mL (Cochran, 1950; Gronewald and Wolpert, 2008; Klee, 1993). All data from the CBT study was initially collected on paper forms. Data was then entered into field or lab data entry programs that were prepared by MEASURE DHS using CSPro Software. Data entry of the CBT Data Collection Form was performed by an ICF International consultant. After data entry and editing was complete, original electronic data files were destroyed and cleaned data files were stripped of all personal identifiers.

### 5.3 Results

*Source of drinking water sample*

Overall, 84 percent of households provided water from an improved source and 16 percent from a non-improved source. Over half of households provided water from a protected dug well or hand pump (51 percent), 14 percent from a water that was piped into their dwelling, yard, or plot, 14 percent from a plastic sack or bottle, and 10 percent from surface water. Table 5.1 presents information on the source of the drinking water sample provided by households.
Table 5.1 Source of household drinking water: Percentage of the distribution of households by source of drinking water according to residence, Liberia Water Testing Pilot 2013

<table>
<thead>
<tr>
<th>Source of drinking water</th>
<th>Households</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urban</td>
<td>Rural</td>
<td>Total</td>
</tr>
<tr>
<td><strong>Improved source</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piped water into dwelling/yard/plot</td>
<td>18.6</td>
<td>0.0</td>
<td>14.3</td>
</tr>
<tr>
<td>Tubewell/borehole</td>
<td>1.9</td>
<td>0.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Protected dug well/hand pump</td>
<td>43.9</td>
<td>74.4</td>
<td>50.9</td>
</tr>
<tr>
<td>Rainwater</td>
<td>4.9</td>
<td>0.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Bottled water/sack</td>
<td>17.8</td>
<td>0.0</td>
<td>13.7</td>
</tr>
<tr>
<td><strong>Non-improved source</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unprotected spring</td>
<td>0.4</td>
<td>0.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Tanker truck/cart with small containers</td>
<td>7.2</td>
<td>0.0</td>
<td>5.6</td>
</tr>
<tr>
<td>Surface water</td>
<td>4.9</td>
<td>25.6</td>
<td>9.6</td>
</tr>
<tr>
<td>Missing</td>
<td>0.4</td>
<td>0.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Number</td>
<td>264</td>
<td>78</td>
<td>342</td>
</tr>
</tbody>
</table>

Contamination levels of household drinking water by source

Over half of households (55 percent) provided drinking water that was categorized as ‘safe’ (an MPN of less than 1 \textit{E. coli} per 100 mL). Fifteen percent of households provided drinking water that tested as ‘low risk’ (an MPN of 1-9 \textit{E. coli} per 100 mL), 14 percent of households provided drinking water that tested as ‘intermediate risk’ (an MPN of 10-99 \textit{E. coli} per 100 mL), and 15 percent of households provided drinking water that tested as ‘high risk’ (an MPN of 100 or greater \textit{E. coli} per 100 mL). Table 5.2 presents the contamination levels of household drinking water overall and by source as measured by the field CBT.
According to the results of the field-based CBT, drinking water was safe in 60 percent of households with an improved source of drinking water and in one in five (23 percent) of households with a non-improved source of drinking water; in contrast, drinking water was high risk in 9 percent of households with an improved source of drinking water and in over half (55 percent) of households with a non-improved source. Bottled water followed by water that was piped into a household dwelling, yard, or plot were the most likely to be safe (83 percent and 74 percent, respectively) and least likely to be high risk (2 percent and 0 percent, respectively). Among households that used drinking water from a protected dug well or hand pump, 56 percent tested as safe and 10 percent as high risk. Only 9 percent of households that relied on surface water for drinking water had drinking water that was categorized as safe by the CBT methodology; 70 percent of drinking water from surface water sources was classified as high risk.
Table 5.2 Contamination level of household drinking water: Percentage of the distribution of households by source of drinking water according to *E. coli* contamination level as measured by the field-based CBT, Liberia Water Testing Pilot 2013

<table>
<thead>
<tr>
<th>Field CBT: <em>E. coli</em> contamination risk category</th>
<th>Safe &lt; 1 <em>E. coli</em> /100mL</th>
<th>Low risk 1-9 <em>E. coli</em> /100mL</th>
<th>Intermediate risk 10-99 <em>E. coli</em> /100mL</th>
<th>High risk ≥100 <em>E. coli</em> /100mL</th>
<th>Total</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Source of drinking water</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Improved source</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piped water into dwelling/yard/plot</td>
<td>60.4</td>
<td>16.0</td>
<td>14.2</td>
<td>9.4</td>
<td>100.0</td>
<td>288</td>
</tr>
<tr>
<td>Tubewell/borehole</td>
<td>73.5</td>
<td>10.2</td>
<td>16.3</td>
<td>0.0</td>
<td>100.0</td>
<td>49</td>
</tr>
<tr>
<td>Protected dug well/hand pump</td>
<td>0.0</td>
<td>20.0</td>
<td>0.0</td>
<td>80.0</td>
<td>100.0</td>
<td>5</td>
</tr>
<tr>
<td>Rainwater</td>
<td>55.7</td>
<td>18.4</td>
<td>15.5</td>
<td>10.3</td>
<td>100.0</td>
<td>174</td>
</tr>
<tr>
<td>Bottled water/sack</td>
<td>15.4</td>
<td>15.4</td>
<td>38.5</td>
<td>30.8</td>
<td>100.0</td>
<td>13</td>
</tr>
<tr>
<td><strong>Non-improved source</strong></td>
<td>83.0</td>
<td>12.8</td>
<td>2.1</td>
<td>2.1</td>
<td>100.0</td>
<td>47</td>
</tr>
<tr>
<td>Unprotected spring</td>
<td>22.6</td>
<td>7.5</td>
<td>15.1</td>
<td>54.7</td>
<td>100.0</td>
<td>53</td>
</tr>
<tr>
<td>Tanker truck/cart with small containers</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>100.0</td>
<td>100.0</td>
<td>1</td>
</tr>
<tr>
<td>Surface water</td>
<td>47.4</td>
<td>5.3</td>
<td>21.1</td>
<td>26.3</td>
<td>100.0</td>
<td>19</td>
</tr>
<tr>
<td>Missing</td>
<td>9.1</td>
<td>9.1</td>
<td>12.1</td>
<td>69.7</td>
<td>100.0</td>
<td>33</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>54.7</td>
<td>14.6</td>
<td>14.3</td>
<td>16.4</td>
<td>100.0</td>
<td>342</td>
</tr>
</tbody>
</table>

**Comparison of field and lab-based results**

As a first step in evaluating the performance of the field CBT, for each set of water samples taken from a single household, the field CBT results to those of the lab CBT and lab MF were directly compared (Tables 5.3 and 5.4). The comparison was made on the basis of the WHO water quality classification scheme. The first point of comparison was the field CBT versus the lab CBT. As shown in Table 5.7, of the 187 water samples that were classified as safe
by field CBT, 127 (68 percent) were classified as safe by lab CBT. Of the 50 samples classified as low risk by field CBT, 17 (34 percent) were classified as low risk by lab CBT. Of the 49 samples that were classified as intermediate risk by field CBT, 11 (22 percent) were also classified as intermediate risk by lab CBT. Finally, of the 56 samples classified as high risk by field CBT, 35 (63 percent) were identically classified by lab CBT.

Table 5.3 Comparison of lab Compartment Bag Test and field Compartment Bag Test results: number of samples with matching \textit{E. coli} contamination risk category enumerated using the lab CBT and the field CBT, Liberia Water Testing Pilot 2013

<table>
<thead>
<tr>
<th>Lab CBT: \textit{E. coli} contamination risk category</th>
<th>Field CBT: \textit{E. coli} contamination risk category</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 \textit{E. coli}/100mL - \textbf{Safe}</td>
<td>127  18  8  4  157</td>
</tr>
<tr>
<td>1-9 \textit{E. coli}/100mL - \textbf{Low risk}</td>
<td>46  17  15  9  87</td>
</tr>
<tr>
<td>10 - 99 \textit{E. coli}/100mL - \textbf{Int. risk}</td>
<td>10  13  11  8  42</td>
</tr>
<tr>
<td>\textgeq100 \textit{E. coli}/100mL - \textbf{High risk}</td>
<td>4  2  15  35  56</td>
</tr>
</tbody>
</table>

The second point of comparison was the field CBT versus the lab MF. As shown in Table 5.4, of the 187 water samples that were classified as safe by the field CBT, 87 (47 percent) were also classified as safe by lab MF. Of the 50 samples classified as low risk by field CBT, 9 (18 percent) were classified as low risk by field CBT. Of the 49 samples that were classified as intermediate risk by field CBT, 24 (52 percent) were also classified as intermediate risk by lab CBT. Of the 56 samples that were classified as high risk by field CBT, 29 (52 percent) were similarly classified by MF.
Table 5.4 Comparison of lab membrane filtration and field Compartment Bag Test results: number of samples with matching *E. coli* contamination risk category enumerated using the lab MF and the field CBT, Liberia Water Testing Pilot 2013

<table>
<thead>
<tr>
<th>Field CBT: <em>E. coli</em> contamination risk category</th>
<th>Safe &lt; 1 <em>E. coli</em> /100mL</th>
<th>Low risk 1-9 <em>E. coli</em> /100mL</th>
<th>Int. risk 10-99 <em>E. coli</em> /100mL</th>
<th>High risk ≥100 <em>E. coli</em> /100mL</th>
<th>Total number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 <em>E. coli</em>/100mL - <strong>Safe</strong></td>
<td>87</td>
<td>13</td>
<td>7</td>
<td>1</td>
<td>108</td>
</tr>
<tr>
<td>1-9 <em>E. coli</em>/100mL - <strong>Low risk</strong></td>
<td>35</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>53</td>
</tr>
<tr>
<td>10-99 <em>E. coli</em>/100mL - <strong>Int. risk</strong></td>
<td>50</td>
<td>18</td>
<td>24</td>
<td>22</td>
<td>114</td>
</tr>
<tr>
<td>≥100 <em>E. coli</em>/100mL - <strong>High risk</strong></td>
<td>15</td>
<td>10</td>
<td>12</td>
<td>29</td>
<td>66</td>
</tr>
<tr>
<td>Incomplete test</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total number of samples</td>
<td>187</td>
<td>50</td>
<td>49</td>
<td>56</td>
<td>342</td>
</tr>
</tbody>
</table>

Lab CBT results were also compared to lab MF, shown in Table 5.5. A difference between this comparison and those previously described is that both tests were performed on a single water sample that was split prior to testing. Of the 157 samples that were classified as safe by the lab CBT, 103 (66 percent) were similarly classified by lab MF. Of the 87 samples classified as low risk by lab CBT, 13 (15 percent) were classified as low risk by lab MF. Of the 42 samples that were classified as intermediate risk by lab CBT, 20 (48 percent) were also classified as intermediate risk by lab MF. Finally, of the 56 samples classified as high risk by lab CBT, 39 (69 percent) were classified as high risk by lab MF.
### Table 5.5 Comparison of lab Compartment Bag Test and lab membrane filtration results: number of samples with matching *E. coli* contamination risk category enumerated using the lab CBT and the lab MF, Liberia Water Testing Pilot 2013

<table>
<thead>
<tr>
<th>Lab MF: <em>E. coli</em> contamination risk category</th>
<th>Safe</th>
<th>Low risk</th>
<th>Int. risk</th>
<th>High risk</th>
<th>Total number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 <em>E. coli</em>/100mL - Safe</td>
<td>103</td>
<td>38</td>
<td>14</td>
<td>2</td>
<td>157</td>
</tr>
<tr>
<td>1-9 <em>E. coli</em>/100mL - Low risk</td>
<td>5</td>
<td>13</td>
<td>64</td>
<td>5</td>
<td>87</td>
</tr>
<tr>
<td>10-99 <em>E. coli</em>/100mL - Int. risk</td>
<td>0</td>
<td>2</td>
<td>20</td>
<td>20</td>
<td>42</td>
</tr>
<tr>
<td>≥100 <em>E. coli</em>/100mL - High risk</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>39</td>
<td>56</td>
</tr>
<tr>
<td>Total number of samples</td>
<td>108</td>
<td>53</td>
<td>114</td>
<td>66</td>
<td>342</td>
</tr>
</tbody>
</table>

Table 5.6 lists the Pearson and Spearman correlations between pairs of the three tests. These correlations measures are appropriate for paired data. The p-values are also listed for the hypothesis tests for each correlation coefficient whose null hypothesis states that the correlation between the two variables is 0. All hypotheses tests conclude that the correlations are statistically significantly different from 0 at the 0.1% level. In order to satisfy normality assumptions, the data were transformed by taking the MPN, adding 1 and taking a square root of that number. The correlation between sqrt(MPN) from lab versus field as well as the correlation between sqrt(MPN) from field and sqrt(count) from standard are both weak to moderately positive when measured either by Pearson or Spearman correlation. However the correlation, both Pearson and Spearman, between sqrt(MPN) from lab and sqrt(count) from standard is moderate to strong and positive. All correlations are found to be significant but not strongly positive.
Table 5.6 Correlations comparing different test methods: number of samples with matching E. coli contamination risk category enumerated using the lab CBT and the lab MF, Liberia Water Testing Pilot 2013

<table>
<thead>
<tr>
<th>Test methods compared</th>
<th>Pearson Correlation</th>
<th>Spearman Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimated Correlation</td>
<td>Estimated P-value</td>
</tr>
<tr>
<td>Field CBT v. Lab CBT</td>
<td>0.67</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Field CBT v. Lab MF</td>
<td>0.52</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Lab CBT v. Lab MF</td>
<td>0.75</td>
<td>&lt;0.0001 *</td>
</tr>
</tbody>
</table>

*Significant at 5% level

The central tendencies of the raw data of E. coli concentration between the field CBT, lab CBT, and lab MF were examined by non-parametric repeated measures ANOVA, also known as a Friedman’s test. This analytical method tests the equality of means for samples that have been matched according to a specific characteristic, in this case, E. coli concentration of samples by test method. Using non-parametric repeated measures ANOVA, there were no statistically significant differences in E. coli concentrations between field and lab CBT results (p-value = 0.7947). However, there were statistically significant differences in E. coli concentrations between field and lab CBT results when compared to the lab MF results (p < 0.0001).

In order to further compare the results of the three test methods on the same scale, a bin analysis was conducted such that data were distributed into four bins as evenly as possible. The association between the level of bin reported by one test with that of another for all pairs of the three tests was compared. The paired nature of the Liberia dataset requires a McNemar Test, specifically the Stuart Maxwell test for 4x4 contingency tables required by the four bins in the analysis. Additionally, the binned data is ordinal (MPN of bin 1 < MPN of bin 2 < … < MPN of bin 4) rather than nominal. The ordinal nature of the paired sample data is taken into account.
when the Stuart Maxwell test is applied. Three tests are completed that provide information on the association of the reported *E. coli* concentration of one test with that of another. Specifically, the probability of being in bin $i$ for one test with the probability of being in bin $i$ for another test is compared. This is done for all four bins (for $i = 1, 2, 3, 4$) and for all pairs of tests. The null hypothesis for each test is listed below:

Test 1. Ho: $P(\text{bin } i \text{ for field CBT}) = P(\text{bin } i \text{ for lab CBT})$ for $i=1,2,3,4$

Test 2. Ho: $P(\text{bin } i \text{ for field CBT}) = P(\text{bin } i \text{ for MF})$ for $i=1,2,3,4$

Test 3. Ho: $P(\text{bin } i \text{ for lab CBT}) = P(\text{bin } i \text{ for MF})$ for $i=1,2,3,4$

When the p-value of one of these tests is significant, the null hypothesis is rejected and we conclude that the probability of being in bin $i$ for one test does not equal the probability of being in bin $i$ for the other test. In other words, the result of rejecting the null hypothesis provides evidence against an association between the *E. coli* concentrations reported by one test with that of the other. Table 5.7 presents the rejection results, p-value, and significance level of each of the three tests. The null hypothesis in Test 1 (p-value of 0.25) is rejected and concludes that there is evidence for an association between the level of MPN reported by lab and that of field. However in Test 2 and 3 we strongly reject the null at the 0.1% significance level. The tests do not provide evidence for an association of the level of *E. coli* concentration reported between standard and field or between standard and lab.
Table 5.7 Results of hypothesis testing for different pairs of test types: number of samples with matching *E. coli* contamination risk category enumerated using the lab CBT and the lab MF, Liberia Water Testing Pilot 2013

<table>
<thead>
<tr>
<th>Test methods compared</th>
<th>Result</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field CBT v. Lab CBT</td>
<td>Failure to reject at all levels</td>
<td>0.7947</td>
</tr>
<tr>
<td>Field CBT v. Lab MF</td>
<td>Reject</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Lab CBT v. Lab MF</td>
<td>Reject</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

*Significant at 5% level

The results of the binned analysis and hypothesis tests are fairly consistent with the results of the non-parametric repeated measures ANOVA conducted with the raw data. There are statistically significant differences between the field CBT and lab MF results, as well as lab CBT and lab MF results. However, no statistically significant differences are found when comparing field CBT and lab CBT. This is also supported by the significant but not strongly positive Pearson and Spearman correlation values when comparing the different test methods and conditions.

5.4 Discussion

Taken together, the results shown in Tables 5.3-5.7 present a complex picture. On the one hand, there is a degree of agreement between each testing methodology, particularly at the extreme ends of the spectrum (i.e., water classified as safe and high risk). On the other hand, the observation that there are statistically significant differences in the central tendencies of *E. coli* concentrations as measured by CBT and by MF suggests there were either differences in the water tested in the field and lab, even when they came from the same household, or that CBT or
MF methodologies used in this study were not reliably robust. Given that only a subset of households from 3 clusters were sampled, it is important to note that these data are neither representative of Liberia as a whole nor of the counties in which the study took place.

Overall, the results indicate the performance of the field CBT and lab CBT was somewhat similar. Specifically, the central tendencies of the MPN values of these two tests were not statistically significantly different as assessed by non-parametric repeated measures ANOVA. In addition, the results of the field and lab CBT showed significant and positive correlation. The similarity in results was achieved despite the fact that holding time for the water samples prior to being tested was much longer for samples tested in the lab (20.5 hours on average) than the field (5.3 hours on average) (USEPA, 2008).

Though, the analysis of the field and lab CBT results when compared to lab MF results showed significant and positive correlation, and statistical differences in the performance of the CBT and MF were not observed in the Peru CBT study, the results of this study indicate statistically significant differences in the performance of the field and lab CBT compared to the membrane filtration results. The central tendencies of the CFU values as assessed by non-parametric repeated measures ANOVA were statistically significantly different than the MPN values for either lab CBT or field CBT. Contamination levels as measured by lab MF were generally higher than by either field or lab CBT. Two hypotheses are proposed to account for the observation:

1) From each household, a single sample of water (~300 mL) was transported to the laboratory. Once at the lab, the sample was divided into aliquots for testing – first, 100 mL was poured off into a bottle for the CBT test; at a later time point, a second 100 mL aliquot was poured off for the MF. The approximately 100 mL of the water sample
that remained in the bag was disposed of. If the 300 mL water sample was not
vigorously shaken before each aliquot was removed, a systematic bias may have been
introduced due to sedimentation of \textit{E. coli} aggregates within the Whirl-Pak® bag,
especially when the sample was kept at 4-10°C prior to analysis; the 100 mL aliquot
that was removed for MF may have had a higher concentration of \textit{E. coli} than that
tested by lab CBT because \textit{E. coli} aggregates that had settled into the bottom of the
bag became re-suspended after pouring out the 100 mL volume for the lab CBT, and
thus more \textit{E. coli} were detected by the MF analysis. However, it should be noted that
approximately 100 mL of water from the sample bag was not tested. If the bacteria
settled in the bottom of the bag, this 100 mL sample would contain a higher proportion
of \textit{E. coli} bacteria relative to the MF sample and lab CBT sample. Because the
remaining 100 mL water sample was not tested for \textit{E. coli} contamination, it is
unknown if the contamination level differed in this aliquot compared to the first 200
mL.

2) On occasion, MF plates were observed in which smears or swarms of \textit{E. coli} appeared.
If the membrane filtration were performed correctly, such a result would be indicative
of a highly contaminated water sample. However, it is also possible to achieve this
result through application of non-optimal laboratory technique in delivering the target
volume of \textit{E. coli} medium to the absorbent pad of the culture dish. Although the MF
protocol called for exactly 2 mL of CBT medium to be added to each absorbent pad,
the laboratory did not follow the standard practice of doing so by measuring the exact
volume with a pipette, but rather measured the volume by pouring manually by hand
and estimating the poured volume by eye. It was observed that in a few instances too
much CBT medium was added to a pad, which caused excess medium to accumulate on the surface of the filter. This may have resulted in progeny bacteria being released from developing colonies, which then spread across the filter and appeared as either extra colonies or a confluence of bacteria growth by the end of the incubation period.

Another possible explanation for why the results of the field and lab CBT did not match the lab MF results concerns the differences in uncertainties in estimating bacteria concentration by each test (Gronewald and Wolpert, 2008). Overall, MPN estimates for bacteria concentration based on a limited number of sample volumes tested results in relatively wide confidence limits for MPN concentrations, especially near the upper and lower MPN concentration detection limits of the test. For example, although the MPN for a CBT in which none of the five compartments is scored as positive is <1 \( E. coli \)/100 mL, the upper 95 percent confidence boundary is 2.87 \( E. coli \)/100 mL; that is, for a CBT result that is classified as safe, there is a 95 percent probability that the true number of \( E. coli \) in the sample is between 0 and 2.87. Similarly, for a CBT in which the largest and smallest compartments (56 mL and 1 mL, respectively) are scored as positive, the MPN is 3.1 \( E. coli \)/100 mL but the upper 95 percent confidence boundary is 11.36 \( E. coli \)/100 mL. Thus, based on the MPN value, the sample is classified as low risk whereas if the true value of the sample is close to the 95 percent boundary (i.e., \( \geq 10 E. coli \)/100 mL), the sample should be classified as intermediate risk. Membrane filtration is also subject to an inherent degree of uncertainty that is not linked to laboratory procedure or error (Gronewald and Wolpert, 2008). Within the context of this study, the true error associated with the MF data is not known. When these uncertainties are taken into account, it is entirely possible that each aliquot of a water sample that is split in half will fall into different contamination classifications.
5.5 Conclusions

This study shows that field surveyors who received a week of training can perform household water quality testing for *E. coli* by the CBT and achieve results similar to trained laboratory technicians in standardized laboratory environments. Both field survey and laboratory water technicians were able to conduct the CBT with ease. While both the field and laboratory CBT results were found to be statistically significant compared to the laboratory MF results, there is strong observational evidence that the reference laboratory did not follow appropriate protocols in separating and conducting the MF analysis. Despite the limited human resources and capacity potentially resulting in overestimation of the MF results, the ability of the CBT to be done successfully under challenging field conditions of Liberia highlights the robustness and applicability of the test in a wide range of settings. The successful incorporation of the CBT within the Liberian DHS, encourages and supports the JMP proposed water quality target for the post-2015 Sustainable Development Goals (SDGs), which includes measurement of *E. coli* concentrations as a fecal indicator of drinking water quality and safety. It is recommended that in future studies such as this; reference laboratories should have adequate and appropriate capacity. This however would be a secondary concern if the CBT is deemed adequate as a microbial water quality test and no parallel sample testing requires reference laboratories. Further field evaluation of the CBT is recommended in order to more fully determine its reliability and field applicability in *E. coli* detection, particularly when compared with a standard method. However, the results of this study demonstrate the ability of the CBT to be reliably and effectively performed in the field by demographic survey staff and give results comparable to the CBT when performed by trained and experienced technicians in a typical microbiology laboratory.
6.1 Introduction

Improvement in water, sanitation, and hygiene (WASH) can reduce diarrhea and other infectious disease risks by up to 40% (Fewtrell et al., 2005; Clasen et al., 2007). Key to this success is the ability to identify unsafe water and inadequate hygiene to implement remedial actions and then monitor effectiveness (Sobsey, 2002). The effectiveness and value of water quality interventions in preventing diarrheal disease depends heavily on acceptability, uptake, and compliance (Clasen et al., 2007; Wood et al., 2012). Household interventions require effort by householders to treat water correctly and consistently, as well as avoiding recontamination during storage or use of untreated sources (Clasen et al., 2007). There are often changes in microbial water quality at the household level because quality may deteriorate through unhygienic handling or ineffective storage, but may also be improved through active intervention or during storage (Bain et al., 2014).

Because microbial contamination of water and other environmental media is not detectable with the naked eye, documenting microbial water quality and demonstrating the link between microbial water quality and potential risk of disease may be a strong enough driver to influence behavior. Thus, a direct test for contamination of household water may be more effective in achieving positive change than solely social marketing messages about safe water (Hamoudi et al., 2012). Even one-time targeted information of this nature can have considerable effects on awareness. A study by Jalan (2009) found that households who were told that their
water was "dirty" (indicating the likelihood of fecal contamination via a presence/absence test for H$_2$S producing bacteria) and were initially not doing any home water purification were 11 percentage points more likely to have begun doing so after seven weeks as compared to households who had not been informed of the test result. More households on average were willing to incur costs (i.e. costs for access to safer water sources, costs of water treatment, costs of safer drinking water storage containers) to get commercially available household drinking water treatments when they saw evidence that they were drinking contaminated water from the results of a presence/absence test for H$_2$S producing bacteria (Hamoudi et al., 2012). These field study results indicate that regular water testing and public information campaigns can not only increase willingness to incur costs for better water sources and effective treatment of drinking water, but potentially also increase demand of safer water from public authorities, at a relatively low cost (Jalan, 2009; Hamoudi et al., 2012).

There are often knowledge gaps at the household level regarding microbial drinking water quality, and there is evidence that increased education is associated with better health practices. Therefore, interventions in WASH knowledge may be appropriate in influencing household WASH practices (Hamoudi et al., 2012). For example, increased education tends to be associated with effort to pursue better health practices associated with WASH. Dasgupta (2004) and McConnell and Rosado (2000) found that in Delhi, India, and an urban area of Brazil, respectively, the education of the household head was significantly associated with the decision to purify water, although there was no report on the magnitude of the effect. The completion of primary education by the female caretaker is also associated with lower *Escherichia coli* hand contamination, and likely more frequent and effective hand washing (Mattioli et al., 2013).
Previous studies observing the effect of water quality testing on household behavior utilized a presence/absence test for \( \text{H}_2\text{S} \) producing bacteria. While \( \text{H}_2\text{S} \) producing bacteria have been demonstrated to be associated with contamination, it is not yet widely accepted as a fecal indicator and measure of potential health risk (Sobsey and Pfaender, 2002). Furthermore, presence absence testing does not provide quantitative data on the magnitude of microbial contamination of the water. The Compartment Bag Test (CBT) is a liquid culture quantal assay that allows for on-the-spot quantitative testing for presence of \( E. \text{coli} \), a widely accepted and commonly used fecal indicator. The CBT is a microbial water quality field test employing a clear plastic bag with internal chambers or compartments of different volumes totaling 100mL to estimate the Most Probable Number (MPN) concentration of fecal bacteria. The CBT can be utilized to not only provide knowledge to households on contaminated drinking water, but also quantify the health risk associated with the microbial contamination, based on WHO health risk categories of \( E. \text{coli} \) concentration in drinking water of low to high risk ranges (0, 1-10, 11-99, 100+ MPN/100mL).

Previous studies have focused on evaluating how the CBT acts as a microbial water quality test in quantifying \( E. \text{coli} \) presence in water. The rationale behind this study was to evaluate the CBT as a tool for microbial water quality education was to see how the CBT would function in a different application. The motivation behind this study was to see if non-technical household users could effectively utilize the CBT and whether its results could influence the knowledge, attitudes and potential practices of people and communities. The purpose of this study was twofold: (1) To obtain qualitative data on health behavior and health education surrounding drinking water and how knowledge, attitudes, and practices may or may not change after understanding of actual microbial quality of drinking water in the household; and (2) To
obtain feedback from users on the CBT as a tool to determine the microbial quality of water. 
The study was done in the urban and peri-urban areas of Mwanza, Tanzania, in collaboration 
with Mwanza Urban Water and Sewerage Authority (MUWASA). The study involved the 
participation of 40 households in a two-day survey regarding their drinking water attitudes and 
practices, and the use of the CBT for microbial analysis of household water quality.

6.2 Methods

Study location and household sample selection

Mwanza, Tanzania, is a residential urban area where the water supply to households was 
not of uniform quality due to the variety of types of community water supplies. There was some 
heterogeneity among the population in terms of their general awareness of sanitation and health 
issues and the city was sufficiently compact to enable implementation of the survey with respect 
to logistics and household access. For these reasons, the study team believed that residents of 
Mwanza would benefit from access to an affordable and easy to use microbial water quality test 
kit. Households were chosen to participate based on MWAUWSA’s previous piped water tap 
survey procedures (MWAWASA, 2008), and included households depending on protected 
springs, shallow wells and boreholes, based on the previous pilot testing conducted by UN-
HABITAT (Davies, 2013). Three different routes were sampled systematically, every fifth 
house, for participating households, based on the main three roads of Mwanza, shown in the map 
in Figure 6.1 Generally, more households were surveyed if the route had more residences. For 
example, more households were surveyed on Route 2: Igoma-Kisesa because this route was more 
populous than other routes.
Figure 6.1 Map of the Town of Mwanza and the three routes on which households were surveyed

Household recruitment and data collection

Working with MWAUWSA, 40 households in the urban and peri-urban areas of Mwanza, Tanzania, were asked to participate in a two-day survey regarding drinking water attitudes and practices and the use of the CBT for the analysis of household drinking water. MWAUWSA provided maps, occasionally transportation, and a translator. On day one, the household head was asked information on household demographics, source and quality of the household drinking water, whether they used any water purification method, and general awareness of the household about water, sanitation, and health issues (Appendix 6). The
household head also analyzed a 100 mL sample of the household drinking water with the CBT under direct supervision, after a brief demonstration of the CBT testing procedure. After the household members conducted the CBT to test their own drinking water, the CBTs were labeled and brought back to the MWAUWSA laboratory at Capripoint for incubation. After incubation of the test for 18-24 hours at 37°C, test results were reported back on day two to the household and a post-water quality analysis survey was conducted regarding water, sanitation, and health attitudes and their reaction to the results of the water quality test. Water quality results were reported back to each household and the explanation of health risk of the water was given based on the WHO guidelines for drinking water quality (WHO, 2011). A thorough discussion on different household water treatment options was also discussed following interpretation of test results.

Data analysis

Quantitative information was tabulated in excel and statistical analysis were performed with SPSS version 21 (SPSS IBM, New York, U.S.A). Perception and knowledge of CBT results on E. coli concentrations in household drinking water was also analyzed as a potential effect measure modifier of people’s perceived health risk from water and to understand the effect of the CBT results on potential behavior change. Qualitative responses were recorded and transcribed in Kswahili, translated to English and coded with Atlas.ti to examine the connections and relationships between perceptions, knowledge of household water quality, attitudes, and practices.
An IRB application for the study purpose and design was submitted and either approved or exempted by the following organizations: UNC-Chapel Hill, the Tanzania Commission for Science and Technology, and the Tanzania National Institute for Medical Research. Households were provided information on the goals of the study and what participation entails and consent was received before the paper and pen survey and interview proceeded. The survey included open-ended questions, results were written directly on the survey, and notes were recorded on another notebook. The interview was recorded with the permission of the household. The survey, notes, and CBTs were labeled with a code that included a route number and a household number but masked household identity.

6.3 Results

Understanding the background of surveyed participants

A total of 40 households were visited and completed surveys on both days. The majority of respondents were female, 72.5% (n=29). Respondents generally reported two or more water sources, usually one for drinking and another for cooking and other uses. Drinking water sources often differed during rainy versus dry season. When categorizing all primary drinking water sources used based on the JMP improved/unimproved classification system, 75% of respondents use improved water sources (n=30) and 25% use unimproved water sources (n=10). A drinking water source is considered primary if the household uses the water source more frequently over other water sources. Figure 6.2 below is a pie chart demonstrating the primary water sources of surveyed participants.
Figure 6.2. Primary drinking water sources of surveyed participant households

*Perception of water safety and treatment on day one*

The majority of families surveyed perceived their water sources to be safe in general for their family and for activities such as hand washing, cooking and cleaning dishes. Safety of water for consumption was perceived to be less safe, with about 65% (n=26) of respondents perceiving their water to be not safe for drinking. Of the households surveyed, 32.5% (n=13) actively treated their household drinking water. The most widely used treatment method was boiling, followed by use of a local water filter. Almost all families have heard of chlorination (i.e. WaterGuard™), however only 5% families surveyed (n=2) used WaterGuard™, a locally available liquid form of free chlorine for household water disinfection. Most households were concerned about adding chemicals like WaterGuard™ to their drinking water due to the smell and taste of chlorine treated water.
Table 6.1 Perception of water safety

<table>
<thead>
<tr>
<th></th>
<th>For use by family members</th>
<th>For hand washing</th>
<th>For washing dishes/cooking</th>
<th>For drinking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safe</td>
<td>80.0 (32)</td>
<td>80.0 (32)</td>
<td>87.5 (35)</td>
<td>65.0 (26)</td>
</tr>
<tr>
<td>Unsafe</td>
<td>7.5 (3)</td>
<td>17.5 (7)</td>
<td>12.5 (5)</td>
<td>22.5 (9)</td>
</tr>
<tr>
<td>Unsure</td>
<td>12.5 (5)</td>
<td>2.5 (1)</td>
<td>0.0 (0)</td>
<td>12.5 (5)</td>
</tr>
</tbody>
</table>

Motivations and barriers to water treatment

Based on interview responses to open-ended questions in the survey, the motivations for treating drinking water arise from health concerns, forthcoming pregnancy, avoiding sickness, saving money by reducing cost of sickness, protecting family, and guidance on treatment from authorities (i.e. MWAUSA, educators, public health campaigns). Sixty-seven percent (n=27) of households reported experienced gastrointestinal problems within the past half a year. Responses to open-ended questions in the survey revealed that barriers to treatment are economics, no perceived risk, lack of technology or training, time constraints, and the idea that destiny is defined and unchangeable. Responses to open-ended questions in the survey also revealed that information on water and sanitation comes from the radio (40%, n=16), MWAUSA (35%, n=14), health officials (27.5%, n=11), public service announcements, NGOs, primary and secondary school, advertisements (i.e. Watergaurd), Maji Week, T.V., and word of mouth.

Perception of water safety and treatment on day two compared to day one

The same question on the perception of water safety was posed to the same person on both days of the survey, and a statistical difference at $\alpha = 0.05$ was found in the perception of safety before and after use of CBT based on conducting a Wilcoxon signed-rank test (p-value = 0.02). On day one, 77.5% (n=31) of participants perceived their water to be “very safe” or “safe”
for drinking, although 45\% (n=18) of the household water samples tested with the CBT were found to have an \textit{E. coli} concentration greater than 10 MPN/100mL, which is deemed by WHO Guidelines for Drinking Water Quality as “high risk” and “unsafe.” On day two, 46.7\% (n=14) of participants perceived their water to be “very safe” or “safe” for drinking. Therefore, the use of the CBT and the observation of visual CBT results dramatically influenced the perception of drinking water safety. Even though drinking water quality was microbiologically safe, with a concentration of less than 10 \textit{E. coli} MPN/100 mL detected by the CBT, there was still a decrease in perception of safety by 88\%-93\% after use of CBT and observation of visual results.

### Table 6.2 Percentage of decrease in perception of safety on day two

<table>
<thead>
<tr>
<th>Household water safety risk on Day Two</th>
<th>Safe</th>
<th>Probably Safe</th>
<th>Probably Unsafe</th>
<th>Unsafe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease in Perception of Safety between Day One &amp; Day Two [100*(1-odds)]</td>
<td>88%</td>
<td>93%</td>
<td>94%</td>
<td>94%</td>
</tr>
</tbody>
</table>

The graph in Figure 6.3 shows the log of perception of water quality on the y-axis and actual water quality as measured by the CBT and categorized according to the WHO drinking water guidelines on the x-axis. Day one perception is shown in blue and day two is shown in pink. Based on the graph, many households perceived their water to be safe on day one, but there is a dramatic decrease in perception of water quality safety on day two. Therefore, a positive result for \textit{E. coli} testing, based on the visual color change, may be useful to motivate health behavior change and encourage households to not only maintain treatment of drinking water, but also improve on safe storage of the water and more hygienic water management to reduce fecal contamination risks.
Reactions to the Compartment Bag Test

The CBT was received with positive reactions. Households noted certain advantages such as being able to “see” microbes, know water safety, avoid sickness, and understand treatment effectiveness. Overall, respondents found the CBT to be user-friendly. On a scale of 1-5 (with 5 being most useful/liked) mean responses were 4.7 and 4.3 respectively. All users say they would recommend the use of the CBT and after seeing results, 87.5% (n=35) said they would change treatment practice. Some households, 10% (n=4), replied that they would not change practice but instead maintain previous practices, because they had water with safe/probably safe levels of *E. coli*.

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3 Water quality categorized as “Safe”, “Probably Safe”, “Probably Unsafe”, and “Unsafe” based on the WHO decimal risk levels for *E. coli* concentrations per 100 mL in the ranges of <0, 1-10, 10-99, and >100, respectively (WHO, 2011).
There are a variety of factors that influence household drinking water treatment, and the motivations of health behavior are complicated. Given that responses are obtained from household users of the test just one day after the testing, the extent to which responses are representative of people’s beliefs, perceptions, and thinking over a longer time period is uncertain. However, through coding translated interviews, a better understanding of motivations and barriers may be elucidated. A network view of codes from the translated open-ended interviews is shown in Figure 6.4. The feelings of not taking responsibility, that things are destined and unchangeable, and economic issues such as lack of money or high costs of access to safer water sources or treatment are barriers and contradict treatment of drinking water. Knowledge and motivation were the main “causes” of water treatment among the respondents.

Figure 6.4 Network view of codes from translated interviews
From the household surveys and interviews, it was found that knowledge is also influenced by and associated with media, word of mouth, education from school, desire for good health, and idea of safety. Motivation encompasses or stems from the desire to protect the family, improve health, and feelings of “the right thing to do.” The CBT can improve knowledge and motivation, which is associated with perception and treatment. From the coding of household interviews, it was found that the CBT recognized the problem of microbial recontamination of previously treated drinking water. The feelings of positivity, improvement and ease of use as a part of the CBT, may lead to the motivation for treatment of drinking water. Therefore, CBT can be utilized as a health behavior and health education tool to influence perception, gain knowledge and encourage household drinking water treatment.

6.4 Discussion

The purpose of this study was to assess whether and how knowledge, attitudes and practices of the head of household around water quality would change after using the CBT and having the opportunity to understand microbial drinking water quality in the context of health behavior and health education. Many barriers to the spread of fecal-oral diseases are related to behaviors such as good personal and domestic hygiene practices and effective water storage and other management practices. Therefore, behavior modifications as well as technical sanitation solutions are necessary to reduce the transmission of excreta-related disease (Carr, 2001). Similar to the findings of Jalan et al (2009) and Hamoudi et al (2012), in our study, one-time targeted information on household water contamination through the use of a microbial water quality test, specifically, the CBT, was shown to have considerable effects on awareness of microbial water quality and household drinking water treatment and storage.
The head of household’s perception that drinking water is safe plays a role in motivating action or as a barrier to decision making about the treatment of drinking water prior to consumption. However, perceptions do not always correlate accurately with presence of fecal indicator bacteria in drinking water (Riesbro et al., 2012). Our results indicate that the use of the CBT to understand microbial drinking water quality at the household level can change perceptions and potentially actions regarding drinking water management. Household interventions on drinking water are important because even water from improved sources is frequently contaminated during collection, transport and household storage and use (Wright et al., 2004; Rufener et al., 2010; Wolf et al, 2014).

The perception of the safety of drinking water is a key indicator of whether water is treated before consumption. Our results indicate that the CBT greatly influences household perception on water quality safety. Between day one, prior to CBT use, and day two, after CBT use, there was a decrease of 88-94% in the perception of water quality safety. There was still a decrease in perception of safety by 88%-93% after use of CBT and observation of visual results, even though drinking water quality was test microbially safe, with a concentration of less than 10 E. coli MPN/100 mL detected by the CBT.

Another objective was to observe whether the CBT was acceptable and user-friendly at the household level. Experience indicates that technology alone is inadequate to secure health gains; without local interest, involvement, and commitment, technologies may remain unused or fall out of use (Carr, 2001). Household members surveyed found the CBT to be simple to use and noted the CBT as a good technology for understanding water safety. All household members surveyed were able to independently test their own household drinking water after a one-time in-person demonstration. For example, in one household a nine year old boy was able to test his
own household drinking water and further explain the CBT steps and significance of CBT results to his elderly grandmother. After using and seeing the results of the CBT, all users (100%, n=40) say they would recommend the use of the CBT to others. The majority of households surveyed, 87.5% (n=35) said they would change water treatment practice, by either start treating drinking water at point-of-use or utilizing improved storage for drinking water. However, households whose water tested as safe/probably safe for levels of \( E. coli \) 10% (n=4), were adamant about maintaining current practices to achieve safe drinking water.

Several households, 27.5% (n=11), reported boiling water before drinking. Yet, in more than half of these households (n=7), \( E. coli \) contamination was found in their drinking water when analyzed with the CBT. A probable reason for this result is the potential for recontamination of treated drinking water during storage or use (Clasen et al., 2008; Rosa et al., 2010; Brown and Sobsey, 2012; Pruss-Ustun et al., 2014). It was the practice of many households to boil a very large pot of water, which was then transferred to another vessel and used for several days. Different household members would obtain drinking water by directly dipping a cup or container into the larger drinking water vessel (as it had no spout for safe dissemination). This action can lead to high potential recontamination risk (Rufener et al., 2010). Seeing the results of the CBT prompted many household members to discuss with family members the maintenance of safe storage and the possibility of recontamination. Therefore, use of the CBT encouraged households to not only maintain treatment of drinking water, but also improve on its safe storage.

There are several limitations to this study including the small sample size and the short time frame of the study, which limit any ability to assess causality. As with any in-person survey, there is the potential for social desirability bias is respondents felt they should report intentions.
to change behaviors. Furthermore, the interpreter assisting with the surveys and interviews is an employee of MWAUWSA, a water provider. Hence there was the potential for responder bias because MWAUWSA is a major organization to advertise public service announcements regarding water safety and treatment. There are also difficulties in quantifying open-ended responses in interview surveys. The interviews were conducted in Kswahili and translated to English, so there may be linguistic misinterpretations or connotations that are misunderstood. Also, there may be cultural barriers that may influence the interpretation of responses and cause inaccuracies in the recording of them. In order to minimize sources of bias, error, misinterpretation and inaccuracy in the recorded data, certain same questions were asked multiple times in different ways and the interpreter’s notes were included with translated interviews in analyzing open-ended responses.

6.5 Conclusions

Based on the observed changes in perception of water quality and safety and the reported ease of its use by household members, the CBT was found to be a potentially useful health behavior/health education tool that should be explored further in future water, sanitation and hygiene interventions. The results of this household survey indicate that the use of the CBT can not only change perception of water safety knowledge, but also increase willingness for use of effective water treatment. Therefore, the CBT is a simple and inexpensive microbial water test that can not only detect and quantify fecal contamination, but also can be used as a public health intervention to result in more accurate perceptions of water quality and safety and potentially influence safer health behavior for water management.
CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

7.1 Summary of Significant Findings

This study was set out to evaluate the Compartment Bag Test (CBT) for the detection and quantification of *Escherichia coli*, the feasibility of its use in low-resource settings, the simplicity of its use for non-technical users, its inclusion and performance within national health surveys, and its utilization and impact as a health behavior and education tool. This study is important in providing further laboratory and field validation for the performance CBT, as a method that overcomes the barriers hindering current methods for microbial water quality analysis in low-resource settings (Bain et al., 2012a; McMahan et al., 2011; Stauber et al., 2014). The results of this research provide evidence to support the promotion of the CBT for its use to facilitate more widespread microbial water quality testing for a variety of purposes in diverse settings. The range of applications for the CBT include operational monitoring for water quality management, microbial water quality analysis in public health surveillance, verification and surveillance of water quality within Water Safety Plans for drinking water, microbial analysis for food and beverage safety, and microbial analysis in disaster preparedness and emergency response. The CBT can be used as a transformative catalyst for water and health interventions and policy change to improve access to clean water and diminish severe health consequences. The CBT can also be utilized as a tool for health behavior and health education for safe water in households and communities.
Previous research on the CBT by McMahan et al. indicated that the CBT yields consistent results when compared to the QT with Colilert MPN system for *E. coli* detection and quantification, and that CBT samples incubated at non-standard temperatures of 27 °C and 44.5 °C gave similar results to a standard temperature of 37 °C (McMahan et al., manuscript in preparation). The sensitivity and accuracy of the CBT when using LTB medium with X-Gluc ranged from 71-82%. However, the study conducted by Stauber et al. indicated that the CBT with an improved chromogenic medium yielded consistent results when compared to membrane filtration for *E. coli* detection (Stauber et al., 2014). Stauber et al. also demonstrated that the use of an improved chromogenic “Hi-*E. coli* medium” can increase the accuracy of the CBT for detection and quantification of *E. coli* by 20.3-33.8% (Stauber et al., 2014). Based on this limited available literature, there was motivation for the CBT with its new and improved chromogenic medium to be further tested for its performance in the laboratory and in the field with different use purposes and conditions. This study sought to evaluate, not only how well the CBT can detect and quantify *E. coli*, but also the feasibility of CBT use by diverse users, in various environments, and with different applications. Research questions included:

1) How well does the CBT detect and quantify *E. coli* in water?

2) Can the CBT be utilized in different low resource settings?

3) Is the CBT user friendly for people who are not trained or skilled in microbial water quality analysis?

4) Can the CBT be included within national health surveys as a method to monitor microbial water quality in households?

5) Can the CBT be utilized as a knowledge intervention and health behavior and education tool to improve water quality awareness and management?
CBT detection and quantification of E. coli in water

In this research, the results for detection and quantification of E. coli concentrations in water with the new chromogenic CBT medium were compared to those for Colilert medium used in the CBT compartment bag and to those for membrane filtration analysis of the same sample waters. Comparing across 600 environmental surface water samples evaluated in a laboratory, the use of the CBT Hi-E. coli medium versus Colilert medium in the compartment bag does not significantly affect MPN outcome in a three-way ANOVA regression (p=0.18). In a pilot field study of the use of the CBT within a Demographic and Health Survey in Peru, the 665 triplicate 100 mL household drinking water samples collected and tested demonstrated that there were no statistically significant differences in results for measured concentrations of E. coli in collected household water samples between the reference laboratory when comparing results of MF and CBT analysis and in field analysis using CBT as determined by the Friedman Test (Nonparametric Repeated Measures ANOVA, p=0.25). Therefore, it was surprising when the 342 triplicate household drinking water samples collected and tested in a pilot field study of the use of the CBT in a Demographic and Health Survey in Liberia demonstrated statistically significant differences between E. coli concentration results as measured by the 1) field CBT analysis and laboratory MF analysis and 2) by laboratory CBT analysis and laboratory MF analysis as determined by a Nonparametric Repeated Measures ANOVA (p<0.0001). These results do not necessary establish that the CBT does not provide comparable E. coli concentration results when compared to MF analysis. Given the circumstances and conditions of household water testing in Liberia, there is evidence to suggest that there may have been issues with the methods of splitting (aliquoting) 100 mL samples from a larger collected sample volume for testing by CBT and MF methods in the laboratory and there were flaws and inconsistencies in
following the required procedures of the MF protocol in the Liberia pilot study. Despite
significant differences in the results for *E. coli* concentrations in household water samples as
measured by the CBT and MF methods in the Liberia pilot DHS survey, all test methods,
specifically field CBT, lab CBT and lab MF, were found to be positively and significantly
correlated with a Spearman’s Correlation ranging from 0.52-0.84. There is a need for further
evaluation of this discrepancy, perhaps with additional statistical analysis with the current data.

To further evaluate the reliability of *E. coli* detection and quantification of the CBT, 559
bacteria isolates were obtained from positive and negative bag chambers of analyzed ambient
surface water samples as culture purified colonies that had been streak plated with sterile wooden
applicator sticks on a differential and selective *E. coli* agar medium. Bacterial isolates were
identified by biochemical assays using a commercial system and by molecular analysis using
commercial systems for uidA qPCR and MALDI-TOF MS. The majority of bacteria speciated
from presumptive positive compartments of the CBT that were identified via molecular and
biochemical methods were *E. coli*, with a false positive rate of 3-11%. Furthermore, the majority
of false positive isolates, 75-88%, were found to be thermotolerant coliform bacteria like species
of *Klebsiella, Enterobacter*, and *Citrobacter*. From different methods of analysis, the false
negative rates were found to be from 0-26%. All false negatives were identified to be *E. coli*. The
false positive identifications of non-*E. coli* coliforms, demonstrate that the CBT provides a
conservative estimate of *E. coli* by marginally overestimating the presence of fecal bacteria
detection in water to protect human health. The accuracy of the CBT was calculated to be 87-
97% based on the results of molecular and biochemical methods utilized to analyze colony
isolates of bacteria from color positive and color negative bag chambers of analyzed surface
water samples.
Feasibility of CBT use by diverse users

The simplicity of the CBT allows anyone with brief training to test their own water, thereby empowering people with knowing if their water is safe or not so that they can determine their own remedial actions. Throughout this research, a variety of users have successfully utilized the CBT to test the microbial quality of water, including: 1) undergraduates at the University of North Carolina at Chapel Hill, 2) field surveyors in the Demographic and Health Survey program Peru, 3) field surveyors in the Demographic and Health Survey program of Liberia, 4) laboratory technicians at the Instituto de Investigacion Nutricional (Peru), La Direcccion Ejecutiva de Salud Ambiental Laboratory (Peru), and Asociación Civil Selva Amazonica (Peru), 5) laboratory technicians in the National Water Quality Control Laboratory of the Ministry of Health and Social Welfare (MOHSW) in Liberia, 6) laboratory technicians at the Capripoint Laboratory of Mwanza Urban Water and Sewerage Authority in Tanzania, and 7) household members in the Town of Mwanza, Tanzania. Users were of various ages, occupations, and education levels. Despite the diversity in background, all users were able to successfully perform and evaluate the results of the CBT after a short demonstration of the steps of the method.

In the Peru pilot study, results demonstrated that field surveyors who received a two-day training can perform household water quality testing for *E. coli* by the CBT and achieve results similar to well-trained laboratory technicians in standardized laboratory environments. In the Liberia Pilot, results demonstrated that field surveyors who received a five-day training program that included training on water quality analysis and the use of the CBT can perform household water quality testing for *E. coli* by the CBT and achieve results similar to well-trained laboratory technicians in standardized laboratory environments. These trainings included how to document
the chain of custody and sample analysis results, how to use an electric and modified power source incubator, and general Demographic and Health Survey protocols. Overall, the steps of the CBT and how to read CBT results were quickly learned and mastered in less than a day.

In the household survey with CBT water quality analysis that was conducted in Mwanza, the household members surveyed found the CBT to be simple to use and noted the CBT as a good technology for understanding water safety. All household members surveyed were able to independently test their own household drinking water after a one time in person demonstration. Even a young boy, aged 9, was able to test his own household drinking water and further explain the CBT steps and significance of CBT results to his elderly grandmother. On a Likert scale of 1-5 (5 being most useful/liked) of being useful and liked, average scores were 4.7 and 4.3 respectively. All surveyed users had no issues with using the CBT to test their own household drinking water and in reading and understanding CBT results, which demonstrates the feasibility of CBT use by diverse users having no prior background or experience in water quality analysis or microbiology.

Feasibility of CBT use in various environments

The design of the Compartment Bag Test was intended to facilitate microbial water quality analysis in low resource settings. This research evaluated CBT performance at different incubation temperatures both in the lab and the field, and in a variety of geographic locations in order to assess the feasibility of CBT use in various environments and settings. Overall, the CBT was found to give generally similar results in detecting and quantifying *E. coli* in water at incubation temperatures between 27 °C and 44.5 °C. However, when evaluating CBT performance at the different incubation temperatures of 27 °C, 37 °C and 44.5 °C in ambient
surface water samples from Chapel Hill, NC, there was a statistically significant difference between MPN results for *E. coli* concentration/100 mL when incubating at 44° C versus 27° C. Yet the magnitude of the effect on MPN concentration of *E. coli* /100 mL was not large, with a sample at 44 °C versus 27 °C having an *E. coli*/100 mL increase of 1.22 MPN. Because the MPN change is slight, with an MPN difference of about 1/100mL, the CBT results from analysis at incubation temperatures between 27 °C, 37 °C, and 44 °C should not greatly influence outcomes for CBT MPN concentrations/100 mL. Therefore, it appears that the CBT can be used to accurately identify and quantify *E. coli* in water samples across various incubation temperatures between 27 °C and 44.5 °C.

In the Peru DHS pilot study of Chapter 4, ambient temperature incubation of the CBT during field sample analysis was used for the region of Loreto. The Loreto region encompasses much of the Amazon jungle in Peru and the average temperatures during the study were 28° C to 31° C. Because the ambient temperatures of the Loreto sites surveyed during the pilot study were between 27° C and 44° C, the field use of the CBT did not require an incubator. It was found that ambient incubation of the CBT in Loreto led to *E. coli* concentration results that were not statistically different from laboratory incubated CBT and membrane filtration *E. coli* concentration results. The use of the modified field portable incubators also demonstrates the feasibility of CBT use in various (and colder) environments. The *E. coli* concentration results of the CBT when heated during incubation using either a methanol fuel burner flame (in Peru) or butane fuel burner flame (in Liberia) were comparable to *E. coli* concentration results for the CBT or membrane filter methods that were incubated with electricity as seen in the Junín region of Peru in Chapter 4 and in the field sites surveyed in Liberia in Chapter 5.

In this research, the CBT was utilized in laboratories in four countries across three
continents. Not all laboratories were of the same type (either academic, research, governmental, or analytical service) or capacity, however all laboratories and their analysts (students, laboratory technicians or analysts, and field survey workers) were able to perform the CBT procedure with ease. The CBT was also conducted in the field in various geographic regions, from environments such as the Pacific coast of Peru, Andes Mountains of Peru, the Amazon jungle of Peru, and African savannas of Liberia and Tanzania. The CBT was also used successfully in urban, peri-urban, and rural environments. In rural environments, field surveyors were able to transport, in a backpack, all the CBT materials for multiple days of water quality testing without difficulty. However, it was more challenging to transport a portable incubator in the various field environments and settings. The electrical incubators and fuel-modified incubators used were much larger than needed for the samples collected by the DHS survey teams. A smaller incubator would be sufficient and would likely facilitate greater ease of transportation.

Feasibility of CBT use in different applications

The results of laboratory evaluation studies conducted by McMahan et al., Stauber et al., and laboratory experiments described in Chapter 3, demonstrate that the CBT can be used successfully as a tool to detect and quantify \( E. \text{coli} \) concentrations in water with performance comparable to standard methods (McMahan et al, manuscript in preparation; Stauber et al., 2014). The simplicity of the procedure allows the CBT to also be utilized in different applications, settings and contexts. The Joint Monitoring Programme (JMP) of the UN, among other numerous international development organizations, desires to know the safety of drinking water at the household level, and national health surveys such as the Demographic and Health survey (DHS) represent an opportunity to examine household water quality on a large scale.
Thus, this research, in Chapters 4 and 5, explored the feasibility of the use of the CBT as a diagnostic tool to assess *E. coli* detection and quantification in household drinking water samples collected and analyzed in the context of a DHS. To date it has remained impractical and logistically challenging to obtain microbial water quality household data at the national level for all countries. This study is the first time a field-portable, quantitative water microbiology test, the CBT, has been included in a DHS and its performance independently verified by analysis of the same water samples by expert laboratories using both the same field water microbiology test, the CBT, as well as a standard water microbiology test, membrane filtration.

The Peru DHS pilot study demonstrates successful incorporation and use of the CBT in the three demographic regions surveyed. The CBT results obtained in the field survey staff were comparable to results obtained by trained analysts in the laboratory using the CBT and a standard membrane filter method. Field surveyors were able to collect, analyze, and document results for CBT analysis of household drinking water samples collected during the day and processed in the late afternoon or early evening. Typically the field CBT results were read and recorded the next day in the late afternoon or early evening. A difficulty arose when survey teams needed to move locations. Some samples would still need to be incubated during travel, which posed a logistical challenge in maintaining incubation conditions of CBT samples while in transit. Because of this challenge, in the case of the Peru DHS pilot study, households visited by the DHS survey teams on the very last day of surveys in a cluster were excluded from household drinking water sampling and analysis with the CBT.

The Liberia DHS pilot study demonstrated that the CBT could enable microbial water quality testing within a DHS, even in a low-income country with limited capacities and minimal non-DHS support. The CBT results obtained in the field were comparable to laboratory CBT
results, thus corroborating that the CBT provides consistency in test results in different environments and applications. While a statistically significant difference in *E. coli* concentrations in household water samples was found between field CBT results and laboratory membrane filtration results, a plausible explanation for this difference was likely errors in maintaining and properly conducting the membrane filtration protocol by the reference laboratory. In the Liberia DHS pilot study, it was feasible to incorporate microbial water quality testing in the household survey. It was even possible to collect household water samples on the last day in a cluster and report back household drinking water safety results from CBT analysis. This may have been feasible because the demographic regions surveyed were urban and peri-urban areas near Monrovia, the capitol city, and because each survey team was equipped with separate transportation for microbial water quality analysis materials and personnel.

Another question addressed by this research is if the CBT can be utilized as a knowledge intervention and health behavior and education tool. The household survey conducted in Mwanza, Tanzania found that there exists an awareness and knowledge gap among many household members in perception of household drinking water safety and actual microbial safety of this drinking water. From the drastic changes in perception of household water quality before and after householder survey, the use demonstration of the CBT and its noted ease of use, the CBT was found to be a potentially useful and easily implementable health behavior/health education tool. The visible color of an *E. coli* positive chamber in the CBT, even if there is only one color positive chamber, can be powerful visible message to change perception on the microbial safety of drinking water. The experimental results indicate that the use of the CBT can not only change perceptions on water safety and convey water safety knowledge, but also increase willingness for effective water treatment actions to improve water quality.
7.2 Implications of Significant Findings

This research was conducted in conjunction with USAID, ICF International, UN-Habitat, country laboratories (NGO, public, and private), and governments. This collaborative effort between academic, public and private international and national organizations provides a unique opportunity to combine science and policy for practice. This extensive and collaborative research is important for stakeholders and members of the scientific community because it validates that the CBT meets the criteria of being not only effective and reliable in quantifying *E. coli* presence and concentration in water samples but is also simple, robust and portable, by providing microbial water quality results comparable to a standard analytical method performed in a laboratory even in rural and rugged areas of a developing country. This is especially important because previous research has demonstrated that there are higher rates of infectious diarrhea found in remote villages than non-remote villages, and people in rural areas often use water sources that are more microbially contaminated than other water used by people in urban areas (Eisenberg et al., 2006; Bain et al., 2014).

While previous laboratory based experiments on the performance of CBT have been conducted, there is the potential for a world of difference between laboratory effectiveness and efficacy in the field. This research is the first to demonstrate the efficacy and applicability of the CBT method for water quality analysis in low-resource settings and its comparability in performance to other currently available and accepted methods. This evaluation of CBT in the field and documentation of observations and experiences in its use and effectiveness in a variety of setting by different users can significantly inform those interested in and attempting to address the challenges of field water quality testing. This evaluation of the CBT also provides information that enables possible solutions to scientific, technical, adaptability, acceptability, and
administrative problems facing the CBT test and other field water quality tests. These results encourage the many applications of the CBT, including its use for management, surveillance and verification of water quality, food and beverage safety, and disaster preparedness and emergency response.

Detection and quantification of *E. coli* by chromogenic broth culture as done using the CBT has clear advantages over other methods to detect and quantify fecal contamination of water. *E. coli* is the preferred fecal indicator of microbial water quality for drinking water because total coliform and thermotolerant or fecal coliform bacteria concentrations in drinking water and its sources are subject to greater variability and uncertainty. This greater uncertainty and variability is due to non-fecal sources of many non-*E. coli* coliforms and thermotolerant coliforms, the ability of some of these bacteria to regrow and enter or produce biofilms, and the propensity of some of these bacteria to clump or attach to particles because of their outer polysaccharide layer or capsule (Edberg et al., 2000; National Research Council, 2004). Use of the CBT as a broth culture methods has advantages over membrane filter methods because clumped or particle-associated bacteria in water will be scored as single colonies in a membrane filter method while such clumped or particle-associated bacteria may disaggregate in a broth culture system using a culture medium containing a surfactant that may help disaggregate bacteria clumps or desorb them from particles.

Because the CBT is inexpensive and simple to perform, microbial water quality monitoring using the CBT could be feasible and realistic in areas where current monitoring does not occur. With the use of the CBT monitoring can be more frequent in order to better detect, in a more timely fashion, the significant changes in microbial water quality that are known to occur over time and in response to events such as rainfall. There can be significant variation in results
from one microbiological sample to another in time and space such that annual sampling at a single location would not be a sufficient predictor of risk. For example, the probability of having at least one failed sample from a site has been shown to increase with the number of samples taken (Riesbro et al., 2012). Therefore, public health can be better protected through increasing the frequency of sampling and analysis, which can be facilitated by the simplicity of microbial water quality monitoring methods, such as the CBT, and more frequent testing (Edberg et al., 2000). Microbial water quality results from the inclusion of CBT in household surveys have provided a direct measurement and therefore a better assessment of the microbial quality of water. This makes it possible to more reliably determine where we stand in the achievement of the safe water access target of the Millennium Development Goals on drinking water, and as the basis for doing microbial analysis of water samples to substantiate the JMP proposed water quality target for the post-2015 Sustainable Development Goals (SDGs), which includes measurement of \(E. coli\) concentrations in water as a fecal indicator of drinking water. Therefore, the availability and use of the CBT can provide quality actionable data on the microbial quality of water for local information, action, interventions, and national policy status and directions.

### 7.3 Research Limitations

This investigative research has begun to fill the gaps in evaluating the CBT for \(E. coli\) detection and quantification in water, with a focus in drinking water, which is supposed to be free of \(E. coli\) in a 100 mL volume of water. However, there are technical limitations of the CBT that are not addressed by this research. A limitation of the CBT is that the upper detection limit is only about 100 \(E. coli\) MPN/100 mL of undiluted water. The limited upper detection limit of the CBT puts some constraints on its comparisons to other microbial water quality tests such as the
Quanti-Tray 2000 multi-well MPN test and the membrane filter test, both of which have the ability to quantify higher concentrations of bacteria before reaching their upper detection limits. Another limitation is that because the CBT has only five compartments, the confidence intervals for the test estimates of MPN concentration of bacteria per 100 mL are somewhat broader than other MPN tests that employ a greater number of discrete sample volumes. However, it is noteworthy that the widths of the confidence intervals of the CBT are rather similar to those for the 10-tube, a 10 mL per tube multiple fermentation tube MPN test for drinking water. These upper detection limit and confidence interval width limitations of the CBT indicate that in situations where all compartments turn positive, indicating exceedance of the highest level able to be detected by the CBT and a high-risk likelihood of containing 100 or more *E. coli* 100mL, the possibility that the *E. coli* concentration is actually much higher than this value should be considered, in order to be more conservative in protecting human health.

A limitation in the laboratory evaluation studies of the CBT is that the sample size of presumptive positives and negative isolates from water samples are not constant among the various experiments due to the loss of viability of some frozen isolates (due to ultra cold freezer failures) and the different timelines of the experiments that led to different durations of isolate storage prior to analysis. However in all molecular and biochemical assays performed, the sample sizes have been greater than 268 samples and provide sufficient statistical power and confidence in the results. Other limitations to this laboratory research are that only environmental or household drinking water samples were evaluated and no pure bacterial strains of *E. coli* or other coliforms of known concentrations in experimentally prepared waters were tested. Such studies would provide more robust information on the absolute recovery efficiency and variability of the CBT Hi-*E. coli* medium utilized in the compartment bag format.
A limitation in field evaluation studies of the CBT is how to account for differences in sample holding times and conditions prior to sample analysis or the reliability and proficiency of analysts in performing sample processing protocols. DHS clusters within regions were often a far distance from the reference laboratories and difficulties in sample transportation generally increased the holding times for samples to be analyzed by the reference laboratories. In the Peru pilot study, some 5% of samples were excluded from all statistical analyses due to excessive holding times of over 48 hours. Also, the average holding time of laboratory samples for the region of Loreto was almost 42 hours. Though the samples were kept chilled in coolers when delivered to the reference laboratory, longer holding time may have contributed to the statistically significant Wilcoxon matched-pairs signed-ranks test results between laboratory CBT and laboratory MF in Loreto. In the Liberia pilot study, there were many different laboratory technicians that processed samples in the reference laboratory, often with different levels of proficiency and experience. Some technicians were of more capable than others and there may have been errors and deficiencies in following the laboratory protocols that led to differences when comparing field and laboratory CBT results with laboratory membrane filtration results.

There are several limitations in the household survey study conducted in Mwanza, Tanzania, including the small sample size of households surveyed and the short time frame of the study. As with any survey study, there is the potential self-reporting bias. Furthermore, the interpreter assisting with the surveys and interviews is an employee of MWAUWSA, the major water utility of Mwanza, which may increase responder’s bias, perhaps because of respondent awareness of the interviewer’s affiliation with the water agency. Because the interviews were
conducted in Kswahili and translated to English, there may be linguistic misinterpretations or cultural connotations that are misunderstood and were not translated accurately or fully.

7.4 Recommendations

While much new information has been developed and CBT performance elucidated through the results of this research, additional research inquiries to assess the effectiveness, performance and acceptance of the CBT for *E. coli* are recommended to address issues that have arisen. Because different physical, chemical and biological parameters of environmental waters can influence water quality, it may be useful to test how specific conditions for these variables may influence the performance of the CBT in detection and quantification of *E. coli* in water. Due to the success of the pilot studies in incorporating the CBT within some regions of the DHS, it is recommended that a future study should look to using the CBT within all regions of the DHS or another national health survey such as the Multiple Indicator Cluster Survey at national scale. Other applications of water quality analysis for *E. coli* using the CBT besides drinking water should be evaluated. Such applications include the analysis of agriculture irrigation water, recreational waters, food safety aqueous solutions such as animal carcass and produce wash water, and hand rinse/hand washing samples, and other hygiene samples such as environmental surface swabs. Because these applications could potentially use a tool such as the CBT for *E. coli* analysis, the CBT should be evaluated for its feasibility performance and validation when applied to these diverse samples and settings. The association between CBT results for *E. coli* concentrations in water and diarrheal disease risks from water could be explored in future research. The portability and ease of use of the CBT facilitates such epidemiological-microbiological research on the relationships between microbial quality of water and human
health risks. Also, while this research shows that CBT results greatly influence user perception and attitudes about water safety, the associations between the CBT results for household water quality and its impact on health behavior actions should be further evaluated. Specifically, the use of the CBT as a health education tool in water management should be studied more systematically and extensively in different cultures and settings, as well as with diverse audiences.

7.5 Conclusions

A key step to ensuring better water quality around the world is having and using convenient, accessible, and reliable water quality testing technology that provides actionable data to make management decisions, supports Water Safety Plans, informs water policies and programs, and stimulates behavior change by people and communities through outreach and education. From the results of this study it is concluded that the Compartment Bag Test (CBT), with minimal training, enables practical, reliable, easy-to-use, actionable water quality testing for fecal microbes, such as E. coli, without the need for a lab, electricity, and supporting equipment.

This research confirms the previous results found by Stauber et al. and shows that the CBT can detect and quantify E. coli in water comparable to standard methods for E. coli detection and quantification, such as the Quanti-Tray Colilert MPN method and membrane filtration (Stauber et al., 2014). Though statistical differences was found between CBT and MF results in the Liberia pilot study, there are several issues concerning failure to follow proper protocol by the reference laboratory that may have contributed to differences. The effectiveness of the CBT for E. coli detection and quantification and its comparability to other E. coli methods,
specifically membrane filtration methods, was demonstrated in both lab studies on ambient surface water and in field studies on drinking waters in diverse settings with different test users.

From the results of this research it is concluded that the CBT method is robust, easy to use by a variety of different users under different circumstances and in diverse settings. The visible results of the CBT were readily understood, accepted, and capable of influencing the perceptions and attitudes of people about the quality of their water. In this research, people from a variety of backgrounds were able to easily use the CBT to test drinking water for \textit{E. coli} presence and concentrations with only minimal training and no prior experience in or knowledge of water quality analysis. The CBT was utilized successfully in a variety of environments and settings, when applied to many different waters, including those in low resource settings and with no access to laboratories. The CBT was incorporated successfully within two national DHS programs on a pilot basis, one in Peru and the other in Liberia, and could be used by field survey staff in the field in the absence of a laboratory.

The CBT has the potential to reliably support widespread and expanded analysis of drinking water quality in a variety of settings for a variety of purposes, including large scale drinking water quality monitoring programs, such as those of the UN JMP. The successful evaluation of CBT for analysis of \textit{E. coli} in drinking water samples from households and communities supports the JMP proposed water quality target for the post-2015 Sustainable Development Goals (SDGs). The post-2015 SDGs include a measurement of \textit{E. coli} concentrations as a fecal indicator of drinking water for monitoring basic and intermediate and safe water service levels, with the safe level defined as no \textit{E. coli} per 100 mL of drinking water and the intermediate level defined as \textit{E. coli} at levels no higher than 10 culturable bacteria/100 mL (Bain et al., 2014). The results of the pilot DHS studies in Peru and Liberia demonstrate the
feasibility of CBT incorporation within national health surveys, which could lead to further evolution and expansion in microbial water quality monitoring, as well as facilitate monitoring efforts for a future drinking water target that includes a direct measure of microbial water quality in households and communities at national scale.

In conclusion, the CBT is an effective and potentially powerful tool to quantitatively evaluate microbial water quality, easily provide water quality analysis training, and positively influence knowledge, attitudes, perceptions and practices regarding water safety and quality. This conclusion is supported by the positive results of the household survey incorporating the use of the CBT to test household drinking water in Mwanza, Tanzania, As documented among household users for the CBT in Mwanza, Tanzania, the simplicity of the test allows anyone with brief training to test their own water, thereby empowering people with knowing if their water is safe or unsafe so that they can determine their own remedial actions. Improved access to simple, actionable water quality testing can drive behavior change and inform decision-making about steps to improve water quality, both in management policies and through on-the-ground water management practices of households and water providers. It is concluded that more accessible and convenient water quality testing could be a transformative catalyst for improving access to safe water and thereby diminish the severe health consequences of unsafe water. By simply making accessible and increasing water quality testing through use of the CBT, safer water for the future can be readily determined and potentially achieved on a more widespread, consistent and sustained basis.
## APPENDIX 1: CBT MPN ESTIMATE OF *E. coli* PER 100ML WATER SAMPLE

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<tr>
<th>Bag</th>
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<th>MPN per 100 mL</th>
<th>Upper 95% CI</th>
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<td>&lt;0.1</td>
<td>2.87</td>
</tr>
<tr>
<td>2</td>
<td>1 0 0 0 0 0</td>
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<td>7.81</td>
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<td>5.64</td>
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</tr>
</tbody>
</table>

* Bolded combinations will statistically occur more common*
APPENDIX 2: TRAINING MATERIALS FOR USE OF CBT IN DHS

Training Manual for Inclusion of Water Quality Testing with the Compartment Bag Test in a National Health Survey

Prepared by the University of North Carolina at Chapel Hill
Gillings School of Global Public Health
December 2012
1. INTRODUCTION

1.1 General Background

The 2011 [Peru] Demographic and Health Survey will be conducted [COUNTRY SPECIFIC INFORMATION]. The technical assistance for [Peru] DHS will again be provided by ICF Macro, USA, and Peru.

The fieldwork will be carried out by a number of interviewing teams. Field teams will generally consist of one field supervisor, one field editor, three female interviewers, one male interviewer, and one or two health investigators. Female interviewers will interview women only and male interviewers will interview men only.

This manual lays out in detail the procedures that will be followed during the [NAME OF SURVEY] fieldwork to obtain the water quality data. These procedures include:

- Obtaining Household water samples and assigning them an identity code
- Transporting the water samples to a location for testing
- Using Compartment Bag Test (CBT) to test water samples
- Reading, recording and transmitting of CBT results in a standard way

1.2 Fecal Indicator Microbe Water Quality Testing

Rationale for Drinking Water Quality Testing for Fecal Microbes

Almost a billion people do not have improved drinking water sources. Even more do not have microbiologically safe water. Information on water contamination such as fecal bacteria presence and levels in water, food and other environmental media helps to inform global programs to reduce infectious diarrheal disease from common exposure sources such as contaminated drinking water and poor sanitation. Annually, 2.5 billion cases of diarrhea and nine million deaths occur among children under five years of age annually. Diarrhea is second only to pneumonia as the cause of these child deaths. An estimated 88% of diarrheal deaths worldwide are attributable to unsafe water, inadequate sanitation and poor hygiene. The inability to properly diagnose diarrhea for treatment and prevention and to determine if water, food, hands and other exposure sources are free of or low in fecal microbes is a major weakness in the ability to tackle these persistent infectious disease problems. Exposure to disease-causing microbes (pathogens) can occur via a variety of routes as shown in the figure below. The route of interest for this survey is ingestion of fecally contaminated drinking water and the diseases of concern are mostly gastrointestinal, such as diarrhea and other enteric ailments caused by bacteria, viruses and protozoan parasites present in fecally contaminated water.
1.3 Microbial Indicators of Water Quality

There are many pathogenic microorganisms responsible for causing disease in humans. These microorganisms can be costly and difficult to detect, isolate and identify from the environment. As a result, routine testing for microorganisms in drinking water consists of tests for microbial indicators of fecal pathogens.

A microbe or group of microbes commonly found in the gastrointestinal tract can serve as a parameter that indicates the potential risk for exposure to enteric pathogens causing infectious diseases by ingesting the water when used for drinking, bathing, or recreational purposes. The best microbial indicators are those whose density or concentration exceeds the concentrations of pathogens and correlates well with infectious diseases associated with fecally contaminated water.

Fecal Indicator Microorganisms

The conventional approach promoted by the World Health Organization and other international, regional, and national authorities to assess the "sanitary" quality of water with respect to fecal contamination is to quantify bacteria commonly present in intestines of humans and warm-blooded animals. These microorganisms have the following desirable characteristics:

1. Present in high numbers/concentrations in fecal matter of warm-blooded animals
2. Methods available to easily detect and quantify
3. Serve as surrogates for pathogens, especially bacteria.

The fecal indicator bacteria typically tested for to determine the amount of fecal contamination in drinking water and the potential risk of diarrheal diseases from ingestion of such water include three related microbial groups: total coliforms, fecal (or thermotolerant) coliforms and *E. coli*. Of these three choices, *E. coli* are considered the most specific indicator of fecal contamination because they almost always come from feces and not other non-fecal sources.

Detecting and Quantifying Fecal Indicator Microorganisms in Water and Other Media

Traditionally, fecal bacteria in a volume of water are detected and quantified by culturing them (allowing them to grow and multiply over time, called incubation) in liquid or solid growth media. The culture media are formulated to select for the growth of the fecal bacteria of interest and to differentiate or distinguish them by the appearance of a unique characteristic, such as a distinctive color change upon incubation for their growth. By using selective and differential
Detecting the presence of a specific kind of fecal bacteria in a water sample by a distinctive color change after culture and incubation simply indicates that one or more of these bacteria were present in that volume of water, but not how many were initially present. The same distinctive color change in the cultured water sample would occur if that volume initially contained any number of these specific bacteria, from one to millions or billions of them. A way estimate the initial concentration of these bacteria in a water sample, is to subject different volumes of the same water sample to culture and incubation. If there are few bacteria of interest in the water being tested, culturing and incubating a series of different volumes of this water, from large to small, will eventually result in volumes of cultured water that contain none of the bacteria of interest. Therefore, there will be no unique color change when these particular volumes of water lacking the bacteria of interest are cultured. By knowing which different volumes of the same water sample show positive growth (color change) and which volumes show no growth (no color change); negative), the concentration of these bacteria in the water can be estimated. This estimation of bacteria concentration based on the different volumes of cultured water sample that become positive (color change) and that remain negative (no color change) is expressed as a Most Probable Number (MPN) concentration, usually per 100 mL volume of water.

### 1.4 A simple approach to Drinking Water Testing for field surveys

Most current tests to detect and quantify E. coli and other fecal bacteria in water and other environmental samples are complex, technically demanding, require a variety of different materials and are costly. To overcome the lack of simple, accessible, portable, self-contained, affordable tests to detect and quantify fecal bacteria in water and other environmental samples, we have developed a compartment bag test (CBT).

This test consists of:

1) A **Sample bottle** in which the water sample is collected.

2) A clear, sterile, disposable **plastic bag containing 5 internal compartments** of different volume that together total 100 mL as the container in which fecal bacteria of interest are cultured and quantified.

3) A chromogenic substrate **culture medium** in which the target fecal bacteria of interest in volumes of cultured water sample are detected and quantified by their production of a
distinctive color change in the medium due to their growth with use of the specific substrate in the medium as food.

4) An external **plastic clip** to isolate the individual internal compartments of water from one another.

5) A **chlorine tablet** for decontamination of the used test kit.

![Figure 1. Components of the Compartment Bag Test](image)

This simple CBT to detect and quantify E. coli or other fecal bacteria in water can be done by almost anyone and fills the previously unmet need for simple quantification of fecal bacteria in resource limited settings.

Additional materials that may be of assistance when collecting, recording, and analyzing water samples:

1) **Disposable Latex gloves:** are used to reduce the to reduce the risk of sample contamination. Gloves must be worn by the health investigator and by anyone else who may assist with the sample collection.

2) **Hand sanitizing with hand gel sanitizer:** prior to sample collection. Hand sanitizing is used to reduce the risk of sample contamination from the hands of those collecting the water sample. Hands of the health investigator collecting the water sample and anyone else who may assist with water sample collection must be sanitized just prior collection.

3) **Bar code labels.** Because the water quality testing is anonymous, bar code labels will be used to identify the household sample (DBS) water samples. These peel-off, preprinted bar code labels are provided on special sheets. Each sheet includes a number of labels with the same bar code. A different sheet will be used for each respondent for whom a DBS sample is collected.
3) **Compartment bag incubator.** After setting up the water test in the CBT, the water samples in CBTs may be placed in a specially designed box, called an incubator, that warms the samples to promote the growth of bacteria while the sample is stored (incubated) for 20-28 hours. The compartment bag incubator is to be used for holding (incubating) water sample tests in CBTs for 20-28 hours in order to grow the bacteria that may be present in the water. They are not intended for long-term sample storage. Instructions will be provided on how to use the incubator. The incubator can be powered to warm its interior by plugging it into an electrical outlet, by connecting it to a battery or by lighting a warmer containing a heating fuel and placing it inside the incubator.

4) **Large sample bags or other waterproof plastic sample carriers.** A large zip-loc bag or other waterproof, flexible plastic container resembling a food transport bag (“lunch bag” will be provided to you for each of the [COUNTRY] DHS sample clusters in which you will work. These large bags will be used to hold the water samples from the cluster during storage and transport.

5) **Plastic bags for biohazardous waste.** These are big bags that are provided to hold all the biohazardous waste materials during the day and will be discarded appropriately after their contents are decontaminated at the end of the day (see Chapter 6).

### 2. STEPS FOR CONDUCTING WATER QUALITY TESTING IN THE FIELD

#### 2.1 Sample Collection

Water samples can be collected from a household or outside, on-plot faucet of a piped water supply, a well, a spring, a cistern or storage tank, a container from a household treatment filter, an on-plot surface water source (e.g., pond or stream), or a drinking water storage container (such as a jerrycan) used by the household for drinking water collection and storage.

Follow these steps for **sample collection**:

1. Inform the household member that you seek their permission to now have them provide you with a sample of water of the kind that they and most members of the household normally drink. If this request in not clearly understood, tell them that you seek their permission and assistance to provide you with a sample of the household water they and most other members of the household would normally drink. If they agree to do this, proceed with the steps below. If they refuse to or are unable to provide you with a water sample, record that no water sample was collected from this household.

2. Identify and record on the water sample form the household drinking water location or dispenser that will be tested. (This could be a faucet of piped water, the spout of a well, a cup or dipper, a container with a water dispensing mechanism, such as a spout, or a vessel from which the water can be poured into the sample bottle. Also record the source or origin of this water, such as from a well or spring, a piped municipal supply (network or distribution system) or a small water storage container (jerrycan, bucket, cistern, or tank).
system), purchased bottled water, or surface water source such as pond, stream or river. The identification of the drinking water location or dispenser and the source of the water will come from the interview with the household member.

3. Ask the household member to collect or dispense the drinking water sample from this specific location as if they were going to drink it. Tell them that they are being asked permission to provide 3 separate volumes of 100 mL each of their drinking water in this way. They will be asked to pour the 100 mL volumes of water into 3 separate sample bottles (each containing sodium thiosulfate).

4. Before collecting the water sample, you and the household member who will collect the sample will sanitize your hands with antimicrobial hand gel. This hand hygiene step prevents cross contamination of samples and also protects you from waterborne microbes that may be present in some of the water samples being analyzed.

5. Prepare 3 water sample bottles by applying sample labels onto their exterior and be sure the labels have the correct cluster ID, household ID, the type of water source (the origin of) the water, location or object from which the water is to be dispensed into the sample bottle, the date, and the time of day.

Have the household member collect or dispense the water from its location as if to drink it and instead have them pour the water into each of the 3 samples bottles from which the lids have been removed. (Be sure to either hold the bottle lids or carefully lay them down in a way that does not contaminate the interior of the bottle lid or its threads.) If need be, hold the open bottle while the household member dispenses the water into it. Make sure to collect at least 100 mL of water, according the fill line on the bottle. Aseptically and carefully place the lid on the bottle mouth and thread it closed to secure it. Once the water samples are collected, place them in a carrier so that they can be safely and securely transported.

### 2.2 Sample Transport and Storage

Follow these steps for sample transport and storage

1. Maintain samples in a safe and secure state for transport and storage. Make sure sample bottles remain closed and do not leak. If a bottle is shaken and its contents leak, attempt to further tighten the lid of the bottle. If this attempt to prevent leakage is not successful, note and record the leak of the bottle on the sample transport form and on the sample bottle itself.

2. Keep track of the number of hours or days that the sample bottles are in transit and storage before the water samples are subjected to analysis. Record on the sample transport log/chain of custody form the conditions under which the samples has been transported and stored as these events occur. (For example, sample transport container from recorded sample collection location was placed in a motor vehicle and driven to the destination where it was to be analyzed; the destination is recorded and the elapsed time since collection and time of analysis is recorded, for example, 8 hours of elapsed time between sample collection and analysis.)
3. Note: If there is more than one sample location and elapsed time period between sample collection and the location and time of sample analysis, record each successive change in location, the estimated temperature conditions and the elapsed time of each of these successive events or locations.

4. Note: Track the conditions for sample collection, transport and storage time for both the sample bottle that is to be analyzed by the member of the survey team, as well as the two sample bottles that are to be analyzed by the “reference” lab. If the sample bottles to be analyzed by the reference lab are placed in the custody of another party (transfer of custody), record the date and time when this transfer of custody occurred, have both parties sign the sample chain of custody/ID form and provide the recipient with the needed sample information and sample ID/tracking information and any need form(s) and labels.

2.3 Sample Testing

Follow these steps for sample testing:

1. **Collect and record** details of 100mL of water sample. Avoid touching inside of sample container or lid. Analyze the sample within 6 hours if possible and no later than 24 hours from time of collection. Protect sample from sunlight and keep it chilled or cool if possible.

2. **Prepare** and sanitize space for analysis. Ensure all materials needed are available.

3. **Mix water sample with growth medium** by dispensing the medium tablet in sample water (without touching tablet with hands). Put the sample bottle lid on and allow 10-15 minutes for the medium to dissolve; periodically swirl to mix.

4. **Open and fill the CBT** with the 100-ml water sample with medium. Each bag has a small white tab located at the top and center of the opening to facilitate holding the bag without touching the bag interior. Gently use the white tabs to pull on sides of the bag to open the bag top and expose all of its compartments. Manually adjust the volumes in each compartment to their fill mark. All water levels should be even.

5. **Seal** the filled compartment bag to isolate the individual compartments of water using a two-piece spring clip by placing U-shaped part of clip against the back of the bag above the water levels in the compartments and snapping the rod-shaped part of the clip in place.

6. **Incubate** the sealed compartment bag for bacterial growth by placing it in an incubator or holding at ambient temperature overnight. Recommendations: For temperatures of 35-44.5°C, incubate at 20-24 hours; for temperatures 30-35°C, incubate at 24-30 hours.

7. **Score and record results:** Examine the cultured water in each separate compartment the incubated bag for the presence (positive/blue) or absence (negative/yellow) of the distinctive color change and record the results for water volume of each compartment as
positive or negative. See the MPN concentration chart for outcomes of positive and negative results.

8. **Decontaminate** by opening the CBT and adding a chlorine tablet. Agitate the bag and let bag stand for at least 15 minutes. After decontamination, pour liquid contents into a sink, toilet or hole in the ground and safely dispose of the empty bag.

**NOTE:** With care, multiple samples can be prepared simultaneously, however, take care to maintain and track sample identity if handling multiple samples at the same time. Avoid sample contamination during collection, transport, storage and analysis. Carefully open each sample container just prior to collection, and close immediately following collection. Do not lay the sample bottles and bag down and avoid touching the mouth or the inside of these containers. Do not rinse the sample containers.

---

**Figure 2. Steps of the Compartment Bag Test**

### 2.4 Recording Test Results

Follow this step and examples for **recording test results**:

On the sample recording sheet, enter or locate the compartment bag number, and then for each of them record if the result is positive (blue-green in color) or negative (no color change)

For example, if all compartments {56, 30, 10, 3, & 1mL} are all blue/green, record a positive on the recording sheet and PDA for 56, 30, 10, 3, and 1mL (See Figure 3a,b,c). If no compartments are blue-green, but instead are the same color as the negative control, then record a negative on the recording sheet for 56, 30, 10, 3, and 1mL. If some compartments are positive (blue-green
color) and some are negative (no color change), record the appropriate result, positive or negative for each volume compartment of 56, 30, 10, 3 and 1 mL, respectively. See examples below.

**Figure 3. Compartment Bag Test with 1 Positive Compartment** (30ml) and 4 negative compartments. Record: 56 mL as negative (0), 30 mL as positive (1), 10 mL as negative (0), 3 mL as negative (0), 1 mL as negative (0).

**Figure 4. Compartment Bag Test with 2 Positive Compartments** (56ml and 30ml) and 3 negative compartments. Record: 56 mL as negative (1), 30 mL as positive (1), 10 mL as negative (0), 3 mL as negative (0), 1 mL as negative (0).

**Figure 5. Compartment Bag Test with 4 Positive Compartments** (56ml, 30ml, 10ml, and 3ml) and 1 negative compartment (1 mL). Record: 56 mL as positive (1), 30 mL as positive (1), 10 mL as positive (1), 3 mL as positive (1), 1 mL as negative (0).
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Figure 5. Entering of Results Data for the Compartment Bag Water Test

### 2.5 Test Decontamination and Disposal

Follow these steps for **test decontamination and disposal**.

1. Put on a pair of protective gloves as provided with the test.

2. Place the compartment bag for which the results have been read and recorded in an upright position.

3. Open a packet of chlorine disinfectant by either tearing along a perforation or by using a pair of scissors to cut it open at its top (above the level of the powdered contents).

4. Remove the two piece spring clip from the bag and set aside but nearby.

5. Open the wire closure of the compartment bag and unroll the folds of the bag.

6. Then, carefully open the bag using the small white tabs located at the top opening and while maintaining the bag upright, pour in the chlorine powder from the open chlorine packet.

7. Close the bag and fold down the yellow closure about 3 times.

8. Place the 2 piece plastic clip across the bag, this time positioned above the top of the liquid AND the top of the internal compartments. This will allow liquid to be completely mixed together with the chlorine, regardless of which chamber the liquid is in.

9. Gently agitate the bag to distribute the chlorine powder and allow it to dissolve completely, as indicated by the no further effervescence.

10. Allow the closed bag and to stand for at least 15 minutes.
11. After 15 or more minutes, remove the plastic clip, unfold the yellow plastic closure and then pour contents of bag into a safe place such as a sink drain, toilet, a shallow hole in the ground (or other liquid disposal container or location)

12. Then, safe dispose of the empty bag as solid waste.

2.6 Reporting sample test results to households and communities

If the survey allows for reporting of results to households and communities of where drinking water was sampled, below are MPN estimate results of the Compartment Bag Test MPN according to World Health Organization health risk categories.

If the results of the water quality test indicate that the water is unsafe or may be unsafe you will also inform the household of the need to consult the safe water brochure that was provided at the time of household visit. That brochure provides information on what to do about poor water quality, its causes, and measures that can be taken to improve it.

Unsafe - all 5 (5 of 5) or 4 of 5 positive compartments

If a water sample has all 5 or 4 of 5 compartments positive (blue-green color change), the water is considered unsafe to drink according to World Health Organization Guidelines. This is because the concentration of \( E. coli \) bacteria is likely to be greater than 100 per 100 ml. Such water should not be consumed without treatment to reduce the bacteria concentration to a lower and safer level. The ways the water can be treated at the household level to make it safe was provided in the flier given to each participating household. When the results are communicated to the household, the household representative can be reminded about the treatment options to make their water safe. Boiling is probably the most widely available and effective option for treatment, but the other treatment methods in the flier are also acceptable. Alternatively, the household can find and use an alternative safe water source, if such sources are known to be available in their community. An example would be bottled water certified to be microbiologically safe and otherwise safe to drink.

Possibly safe - 3 of 5 positive compartments

If a water sample has 3 of 5 compartments positive (blue-green color change), the water is considered possibly safe to drink according to World Health Organization Guidelines. Such water can be consumed if no other water that is safe is available. This is considered only possibly safe because the concentration of \( E. coli \) bacteria is likely to be between 10 and 100 per 100 ml. Such water should not be consumed indefinitely without treatment to reduce the bacteria concentration to a lower and safer level. It may be consumed for a short time until further treatment of the water can be organized or an alternative safe source becomes available. The ways the water can be treated at the household level to make it safe was provided in the flier given to each participating household. When the results are communicated to the household, the household representative can be reminded about the treatment options to make their water safe. Boiling is probably the most widely available and effective option for treatment, but the other
treatment methods in the flier are also acceptable. Alternatively, the household can find and use an alternative safe water source, if such sources are known to be available in their community. An example would be bottled water certified to be microbiologically safe and otherwise safe to drink.

*Safe: 0, 1 or 2 positive compartments*

If a single water sample has no (0), 1 or 2 of 5 compartments positive (blue-green color change), the water is considered safe to drink according to World Health Organization Guidelines. This is because the concentration of *E. coli* bacteria is likely to be less than 10 per 100 ml. Such water can be consumed without further treatment. However, if a series of water samples from a central community water supply, a community well, or other community system yield positive results (compartments) in the test, it is recommended that local authorities be informed and that further investigation of the safety and integrity of the water system be investigated. Community water systems are expected deliver water free of *E. coli* or other fecal bacteria and should give consistently negative results (no positive compartments) in the water test.

### 3. COMPLETING QUESTIONNAIRES AND OTHER TESTING DOCUMENTS

As the technician, you will be responsible for the accurate recording of information that will be used to track the outcome of the testing procedures during the survey and provide results of the water quality test to the household member who provided the water sample. The following are the documents for which you will be responsible during the fieldwork:

- Water quality information page in the Household Questionnaire: You will enter the required information on this document.
- Safe Drinking Water Brochure: You will provide a copy of this brochure to the respondents of the households you visit and recruit. You will answer any questions the respondent may have about the information in this brochure or, if you cannot answer their question, if will direct them to other sources of information who can answer their question(s) about safe water.

#### 3.1 Identifying Eligible Respondents

The first step in the testing process will be to identify members of the household who can consent to the water quality testing. For all eligible women (age 15-49 years), eligible men (age 15-49 years), water quality information will be recorded on the **Water Quality section** (Figure 3.1) of the Household Questionnaire

**It is the responsibility of the interviewer** to identify and record all the eligible respondents for participation. Health Investigators will get the household questionnaire with information filled in by the interviewer. However, Health Investigators should verify the information by reviewing the following from the Household Schedule:
- Column (1) Line number
- Column (2) Name
- Column (4) Sex of household member
- Column (7) Age of household member
- Column (9) Identification of eligible women, (women age 15-49)
- Column (10) Identification of eligible men, (men age 15-49)
- Column (11) Identification of eligible children, (children under age 6)

Figure 3.1

For women, verify that the line number and name of all women age 15 to 49 are listed in Question ( ). For men, verify that the line number, name, and age of all men age 15 to 49 are listed in Question ( ).

The following are important points to keep in mind when identifying eligible respondents and the appropriate water sources to test:

1) All women and men who fit the appropriate age categories are eligible to help provide a water sample for testing, whether they are usual residents in a household or visitors.
If you have any questions about a woman’s or a man’s eligibility to help collect a sample of household drinking water, ask the team supervisor.

2) **In principle, eligible consenting respondents should be asked to provide a sample of the drinking water being used in the home most often and by most members of the household at the time of visit.**

3) **Never alter any responses on the Household Questionnaire without consulting the interviewer (if you were not the interviewer for the Household Questionnaire).** For example, you may be a health investigator collecting DBS from a male respondent (after the male interview), while a female interviewer on your team conducted the Household/female interview.

### 3.2 Obtaining Informed Consent for the Testing

One of the primary tasks before testing is to explain the purpose of the testing to eligible respondents and to obtain their consent before collecting any water quality samples. In order to ensure that these individuals can make an ‘informed’ decision about the testing, the [COUNTRY] DHS questionnaire includes statements which you will read as appropriate. These ‘informed consent’ statements (Figures 3.4 and 3.5) include the following basic elements:

1. A description of the objectives of the water quality test
2. Basic information on how the test will be conducted
3. Assurances about the confidentiality of the results
4. A specific request for permission to collect the sample.

You must read the informed consent statements to an eligible respondent age 18 and over and obtain the respondent’s consent to the testing before any water quality sample collection is done. In all cases, you must record the outcome of the consent request before asking the respondent to provide a drinking water sample.

You must sign your name to indicate that you read the consent statement to the adult and have recorded their response accurately.

The following are some key points to remember in obtaining consent for the testing:

1. **Read the applicable consent statements to each eligible respondent exactly as they appear in the questionnaire.** When you arrive at the household and begin talking about the water quality tests with an eligible respondent, you may informally discuss many of the items included in the informed consent statement. However, before beginning the collection of the water sample for testing with any respondent, you must still read the informed consent statements exactly as they are worded in the questionnaire. If you feel that the respondent may find the statements repetitive, tell him/her that you are required to formally read the statement to ensure that respondents are given all the appropriate information. There will be only one respondent per household from who permission is sought to collect the water sample. The preferred respondent is the household member
generally responsible for household water management and related water, sanitation and hygiene household duties, such as child care, cooking and cleaning

2. **Be sure that you read the informed consent statements clearly.** Practice reading the consent statements so that you become comfortable reading them in a clear, natural manner. Avoid using a monotone tone when reading the statements or reading them so rapidly that they cannot be understood.

3. **Always request consent for the Water quality tests from an adult member of the household.** Be sure the respondent knows that the preferred household member to provide the water samples for testing is the one responsible for household water management and perhaps related household water, sanitation and hygiene activities. However, another adult knowledgeable about drinking water conditions and practices in the home can be the consenting participant if the other household member generally responsible for household water management is not available.

4. **Never attempt to force or coerce consent.** It may take tact and patience to overcome people’s fears about having water quality collected for testing. Take your time in trying to convince respondents who are uncertain about the testing to grant their consent. Some respondents may have questions or want to discuss the procedures before giving consent. Patiently respond to all questions.

5. Some respondents from whom you are seeking consent may be reluctant to allow any drinking water collection for testing without consulting someone not present at the time of your visit (such as a woman who may want to consult her husband before giving permission). **In such cases, make an appointment to return to the household later at an agreed upon time.** If you believe it will help, ask the team supervisor to visit a household where eligible respondents express fear or reluctance to be tested.
4. BIOHAZARDOUS WASTE DISPOSAL

Any material coming in contact with a potentially contaminated water (sample collection containers, CBTs, etc.) is considered to be biohazardous. Safe disposal of such material is very important to prevent the transmission and spread of various waterborne diseases among survey personnel and within the study community. Biohazardous waste has to be collected in a special container during the water sample collection and analysis, securely stored and transported, and safely disposed at the end of each day of fieldwork.

If possible, commercially available biohazardous waste disposal containers should be used for biohazardous waste disposal. These types of containers are red and have a special logo warning about biohazardous content. They can be securely closed for safe storage and transportation during the fieldwork. Whenever possible, the biohazard bags should be taken to health facilities, which employ standard procedures for biohazardous waste disposal. If this is not possible, survey staff can complete daily biohazardous waste treatment and disposal with chlorine, according to the procedures described.

In this procedure for analysis of water, all biohazardous materials are decontaminated by treatment with free chlorine to decontaminate and sanitize them for safe disposal. The methods for such decontamination are described in the procedures above. A summary of the biohazardous waste, its treatment and its safe disposal are given below.

1. Sample water sample bottles. After use, fill water sample bottles with clean water, such as tap water or another source of drinking water, add a chlorine tablet, secure the bottle cap and allow to stand upright for 15 minutes.

2. After 15 minutes, invert the bottle and allow to stand an additional 15 minutes. Then, remove the cap, pour out the contents of the bottle in a safe place (e.g., toilet, latrine, waste drainage ditch or on the ground (open land with porous soil).

3. CBT. After performing the analysis of a water sample in the CBT and examining and recording the results, treat the contents of the CBT with a chlorine tablet as described above. After chlorine treatment, the contents of the decontaminated CBT can be disposed of in a toilet, latrine, waste drainage ditch or on the ground (open land with porous soil). The bag can be disposed of with other solid waste, as for any used plastic bag.

4. Disposable gloves. After wearing a pair of disposable gloves to decontaminate the CBT, remove the gloves and place them in a plastic bag or bucket containing a solution of about 500 PPM chlorine bleach (5 chlorine tablets). Allow to gloves to be in contact with the chlorine solution for 30 minutes. After 30 minutes, pour out the chlorine solution in a safe place (toilet, sink, latrine, waste ditch or on the ground). Then dispose of the gloves with solid waste. The chlorine bleach solution is made by adding 1 part of household bleach (5 to 6% hypochlorous acid) to 100 parts of tap water or other clean water, or 5 chlorine tablets to 100 parts tap water.
5. Plastic clips for the CBT. After using a plastic clip to secure a CBT, remove the clip and place the two parts in a plastic bag or bucket containing a solution of about 500 PPM chlorine bleach. Allow the clip pieces to be in contact with the chlorine solution for 30 minutes. After 30 minutes, pour out the chlorine solution in a safe place (toilet, sink, latrine, waste ditch or on the ground). Then recover the clip, rinse with water and dry for future use. The chlorine bleach solution is made by adding 1 part of household bleach (5 to 6% hypochlorous acid) to 100 parts of tap water or other clean water.

It is the health investigator’s responsibility to ensure proper disposal of biohazardous waste. It is unacceptable that the materials used during the testing in one fieldwork cluster are carried by the team to the next cluster. Biohazardous materials must be destroyed at the end of the day.
APPENDIX 3: INSTRUCTIONS FOR USE OF MODIFIED INCUBATOR

Instructions on the use of the Modified Incubator

Parts of the Modified Incubator

Incubator

(inside incubator) Butane Burner

Butane Canister (not pictured)

(inside incubator) Temperature Logger

Fitted Caps x3 (to allow for ventilation/no ventilation)

Butane Adaptor

Gas Control Valve with Tubing
Set up of the Incubator for use with Electricity

1. Place all three fitted caps on.
2. Plug the cord into an outlet.
3. Push the “on/off” button, a temperature reading in a blue light will come on at the top of the incubator.
4. Adjust temperature to 98°F.
5. Allow for 30 to 45 minutes for the incubator to warm up.
6. Press the red button on the top of the temperature logger for at least 1 second when samples are placed in the incubator.
7. When samples are taken out of the incubator to be visually scored, press the red button on the top of the temperature logger for at least 1 second.
8. To turn off incubator, push the “on/off” button and unplug.

Set up of the Incubator for use with Butane

1. Remove the three tubing caps for ventilation.
2. Place the butane cannister inside the adaptor, screw the adaptor pieces together, this will pierce the butane cannister. Leave cannister in place until it is empty. The cannister should be sufficient for about 4 days. The cannister will sound like there is no liquid inside when it is empty and will also feel lighter. To change cannisters: make sure the adaptor is not connected to the gas line and make sure the butane cannister is empty, unscrew the adaptor and remove the empty cannister, replace with a new cannister as described previously.
3. Locate the gas control valve and tubing assembly. Make sure the valve is in the OFF position (turn snuggly in the clockwise direction but do not force it).
4. Screw the gas control valve snuggly onto the butane adaptor (with the butane cannister inside the adaptor).
5. Connect the gas control valve tubing to the access port on the right rear side of the incubator by pushing the access port sleeve back (toward the incubator), fully inserting the gas line fitting, and releasing the sleeve. Make sure the gas line is secure.
6. Turn on the butane gas by turning the valve on the butane adapter in the clockwise direction.
7. Inside the incubator, move the burner switch firmly to the ON position.
8. Ignite the butane by pressing the START button repeatedly until you see a small blue or orange flame. It may take 15 – 30 seconds of pressing the START button before a flame is observed. If, after a minute or more, you are unable to start the burner, recheck the gas valves to make sure they are turned on and that the gas cylinder still has gas in it.

9. Allow one hour for the incubator to warm up.

10. Press the red button on the top of the temperature logger for at least 1 second when samples are placed in the incubator.

11. When samples are taken out of the incubator to be visually scored, again press the red button on the top of the temperature logger for at least 1 second.

12. When finished with the incubator, move the burner switch to the OFF position and turn the gas control valve anti-clockwise until snuggly closed.
## APPENDIX 4: LIBERIA CBT FIELD DATA COLLECTION FORM

1 April 2013

<table>
<thead>
<tr>
<th>PLACE NAME</th>
</tr>
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<tbody>
<tr>
<td>NAME OF COUNTY</td>
</tr>
<tr>
<td>NAME OF HOUSEHOLD / ICAD</td>
</tr>
<tr>
<td>LDHS CLUSTER NUMBER</td>
</tr>
<tr>
<td>HOUSEHOLD NUMBER</td>
</tr>
</tbody>
</table>

### IDENTIFICATION

| INTERVIEWER VISITS |
| --- | --- | --- | --- |
| DATE | 1 | 2 | 3 | FINAL VISIT |
| INTERVIEWER'S NAME | 
| RESULT* | 
| NFXT VISIT DATE | 
| TIME | 
| TOTAL NUMBER OF VISITS | 

*RESULT CODES:
1. COMPLETED
2. NO HOUSEHOLD MEMBER AT HOME OR NO COMPETENT RESPONDENT
   AT HOME AT TIME OF VISIT
3. ENTIRE HOUSEHOLD ABSENT FOR EXTENDED PERIOD OF TIME
4. POSTPONED
5. REFUSED
6. DWELLING VACANT OR ADDRESS NOT A DWELLING
7. DWELLING DESTROYED
8. DWELLING NOT FOUND
9. OTHER (SPECIFY)
APPENDIX 5: LIBERIA DHS SAFE WATER BROCHURE

**What are some methods for making water safe to drink?**

There are many methods of treating water to make it safe to drink. A few methods are listed here that describe how to remove bacteria from the water.

**Boiling:** Households can disinfect their drinking water by bringing it to a rolling boil and letting the water boil for one minute.

**Chemical disinfection:** Using chlorine is the most common method of treating drinking water with chemicals. When using bleach, add 8-16 drops of household liquid bleach to one gallon of water. Mix well and wait 30 minutes or more before drinking. You can also use Pur or Watergaurd to make the water safe to drink.

**Filtration:** Water filtration is another option to purify water. There are many types of filters available. Ceramic filters are commonly used. They have small pores and are often coated with silver to kill bacteria.

**How to wash your hands**

Handwashing can also reduce illness experienced by people in your household. Follow the simple instructions to properly wash your hands:

- Wet your hands with water. If possible use running water.
- Apply liquid, bar, or powder soap and lather well.
- Rub your hands vigorously for at least 20 seconds. Scrub all surfaces, including the backs of your hands, wrists, between your fingers and under your fingernails.
- Rinse well and dry your hands

<table>
<thead>
<tr>
<th>TEST RESULTS</th>
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<tbody>
<tr>
<td>SAFE TO DRINK</td>
<td>no <em>E. coli</em></td>
</tr>
<tr>
<td>MAYBE SAFE TO DRINK</td>
<td>1-9 <em>E. coli</em> in a small glass of water</td>
</tr>
<tr>
<td>MAYBE UNSAFE TO DRINK</td>
<td>10-99 <em>E. coli</em> in a small glass of water</td>
</tr>
<tr>
<td>UNSAFE TO DRINK</td>
<td>100 <em>E. coli</em> in a small glass of water</td>
</tr>
</tbody>
</table>

**What do the water test results mean?**

**SAFE TO DRINK:** There are no *E. coli* bacteria present in the water sample. Your water is safe to drink without further treatment.

**MAYBE SAFE TO DRINK:** There are a few *E. coli* bacteria in your water. You should consider treating your water before drinking it.

**MAYBE UNSAFE TO DRINK:** *E. coli* bacteria are in your water. It is possible that the water could be unsafe to drink and cause illness if you drink it. You should consider treating your water before drinking it.

**UNSAFE TO DRINK:** There are many *E. coli* bacteria are in your water, and the water is not safe to drink. The water should be treated before drinking it.
What are some ways to safely store my drinking water?

Even if water is safe to drink when it’s collected, it can become contaminated if stored improperly. To make sure that water is stored safely, remember to:

- Cover containers with a lid;
- Use containers with narrow openings and dispensing devices such as spouts, taps, or spigots;
- Sanitize containers before filling them with safe water

How are containers sanitized?

- Add 5 milliliters (64 drops) of household liquid bleach to 1 quart or 1 liter of water.
- Pour the solution into a clean storage container and shake well, making sure that the solution coats the entire inside of the container.
- Let the container sit for at least 30 seconds, and then pour out solution.
- Air dry the container or rinse it with clean water that has already been made safe.

National Water Quality Control Laboratory
Ministry of Health and Social Welfare

Name of Household Head: ______________________
Date: _____________________________

National Water Quality Control Laboratory, of the Ministry of Health and Social Welfare, and Liberia Institute of Statistics and Geo-Information Services (LISGIS) are conducting a study on water quality. The study will help us identify whether people are drinking safe water in Liberia.

We appreciate that we have had the opportunity to test your household’s water. Thank you for your cooperation.

Please look inside for the results of the water testing.
APPENDIX 6: CBT KNOWLEDGE INTERVENTION SURVEY

Evaluation of the Compartment Bag Test for Microbial Water Quality Monitoring in Mwanza, Tanzania

Introduction & Informed consent

[To household head]

Hello. My name is ____________ [& introduction of other team members]. We are gathering information on the use of the Compartment Bag Test (CBT) for microbial water quality monitoring to determine user feasibility and how they feel about the water quality testing. May I speak to the person of the house who is responsible for the household drinking water?

[To household head responsible for drinking water that will answer the survey]

Hello. My name is ____________ [& introduction of other team members]. The University of North Carolina (UNC) Gillings School of Global Public Health is conducting a research study to evaluate water microbial quality understanding and the effectiveness of the Compartment Bag Test for household water testing. We would like to talk with you today for about an hour to better understand your household’s water supply, practices, and opinions. In addition, we would like to take a sample of the water that you use for drinking to test its quality with you. We will not reveal your identity in any report of this survey. You are not obliged to take part in this survey and you can refuse to have a conversation with me, but I would like to ask you to help me in doing this work. Would you like to take part in this survey? If you agree to take part, we will ask you to do the following things:

First, we will ask you questions about you and your family members, your water supply, how you handle water, and your opinions about these things. At the end of the questions, we will take a sample of water from your water source and your household container to test it for purity, together. These two activities together should take about 1 hour.

Second, with your permission, someone from the research team will return to visit you tomorrow to ask you a few questions about your use of the water test and give you the water quality results from today. This returning visit should take approximately 30 minutes.

Your decision to take part is completely up to you. If at any time during the questionnaire you decide that you no longer wish to take part, you may withdraw with no penalty. All information you give us will be kept confidential to the extent possible. The names of you or your family members will not appear on any report of this project. You may also decide to stop participating in this study at any time in the future, with no penalty.

Your taking part may help improve the design of a new water quality field test that can improve the drinking water situation in your community. The only anticipated risk to you by participating in this study is the potential bother of the time to respond to questionnaires at each visit. If you do not like one of the questions though, you do not have to answer it.

Do you have any questions? If at any time during the project you have questions about the project or about your rights as a person in a research project, you may speak to __________________________.
Would you like to take part in this research study? [If yes]: I am now going to read you a statement, and if you agree to it, I and Mr./Ms. ________ will sign this paper to confirm that.

**Agreement to Participate**

By signing below, I indicate that I have had the study and consent form verbally presented to me in a language I understand. I have had the opportunity to ask, and have had answered, all my questions about this study. I voluntarily agree to participate in this research study.

_________________________________________   _______ ______________
Signature/Mark of Research Subject    Date

_________________________________________
Printed Name of Research Subject

_________________________________________   ________ ____________
Signature of Interviewer/Interpreter    Date

_________________________________________
Printed Name of Interviewer/Interpreter

_________________________________________  ________ ____________
Signature of Person Obtaining Consent   Date
(Project Manager)

_________________________________________
Printed Name of Person Obtaining Consent (Project Manager)
**Day ONE Interview**

**HOUSEHOLD DATA**

*Respondent gave verbal consent to be interviewed and signed informed consent*

<table>
<thead>
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</tr>
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<tr>
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<table>
<thead>
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<tbody>
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<td>Interview Date</td>
<td><em><strong><strong>/</strong></strong></em>/_____</td>
</tr>
<tr>
<td>Household Code</td>
<td>_____ _____ _____ _____ _____</td>
</tr>
<tr>
<td>Name of Respondent</td>
<td></td>
</tr>
<tr>
<td>Respondent Sex</td>
<td>M / F</td>
</tr>
<tr>
<td>Respondent Age</td>
<td>_____</td>
</tr>
</tbody>
</table>

**UNDERSTANDING BASELINE, PROBLEMS, & BARRIERS**

*Drinking Water Source*

1. What water source do you use to get water for the household currently? *[Do not read options; Record all that apply]*
   a. Tap inside the house
   b. Tap outside the house
   c. Shallow (hand-dug) well, lined
   d. Shallow (hand-dug) well, unlined
   e. Deep (drilled) well
   f. Lake or pond
   g. River, stream, or canal
   h. Rainwater
   i. Purchased water
   j. Other(s) [record response] ____________________________________________
      ____________________________________________
      ____________________________________________

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2. Do you think this water source is safe for you and your family?  Y / N

3. Is this water safe enough to wash hands in?  Y / N
   - do dishes?  Y / N
   - cook?  Y / N
   - drink?  Y / N

Water Treatment
4. Do you do anything to try to make the water safer before you wash your hands?  Y / N
   - do dishes?  Y / N
   - cook?  Y / N
   - drink?  Y / N

If Y, how? [Do not read options; Record all that apply]
   a. Boiling
   b. Chlorination with bleach
   c. Other chemical [record response] _________________________________
   d. Ceramic water filter
   e. Biosand filter
   f. Other sand or granular medium filter
   g. Letting the water “settle” in the container
   h. Coagulation using alum or other coagulant [record response] __________
   i. Tap water is already treated
   j. Does not treat water
   k. Other treatment [record response] _________________________________

5. [If water is treated] Do you think your actions make the water safer?  Y / N

If N, why do you do it? [Do not read options; Record all that apply]
   a. Water is dirty
   b. Water is not safe to drink
   c. Water has bad taste
   d. Was told water should be treated
   e. Other reason [record response] _________________________________

Problems
6. Are you satisfied with your current drinking water? (i.e. water access, flow, safety) [Prompt if necessary]

7. Has water ever made you or your household/friend(s) sick?  Y / N

If Y, how often?
8. Do you know which water sources in your neighborhood or village are safest? Which ones are generally the most dangerous/make people sick?

UNDERSTANDING INCENTIVES & ROLE OF INFORMATION

Motivation
9. What motivates you to act on [refer to previously stated problem(s)] __________ of water safety in your house (facilitators)? Do you perceive this to work?

Barriers
10. What, if anything, prevents you from acting on problems of water safety in your house (barriers)? (i.e. cost, time, no perceived risk) [Prompt if necessary]

Role of Information
11. What information do you get? (i.e. media, posters, advertisements, sales in stores/kiosks, word of mouth, NGO aid workers) [Prompt if necessary]

Activity: Describe an example of a health message they have seen lately - what it was about, what caught their attention, and internally how did it make them respond? Possible to get an example of 1 influential and 1 non-influential and then follow up with questions as to why?

12. Specifically for water and health - what message have you received in the past, how have you responded?

13. Does information on water and health help you to choose your actions in solving the water safety problem in your house? If so, what type(s) of information do you like/respond to?

REATIONS TO THE COMPARTMENT BAG TEST

This is the Compartment Bag Test. This test can provide information and generate knowledge, similar to [name something mentioned prior] ____________. It is a test that can detect and quantify bacteria in water. Bacteria are small living organisms that can be in your water, some, not all, of which can make people sick.

14. [Based on response from Q6]
   Y → You had mentioned water has made you sick or other(s) you know, what do you think caused it?
   N → You had mentioned that you and other(s) did not get sick from water, what do you
think prevents sickness?

(i.e. bad smells, cloudiness, dirt, bacteria) [Prompt if necessary]

[Fill in necessary information on microbiology to give background on the Compartment Bag Test based on their responses, enough so they can understand what the test is about. Ask permission to take a water sample and show/teach them how to use the bag test.]

May I take a sample of the water that you drink and we can use the Compartment Bag Test together? First, I will demonstrate the steps of the test with you observing and then you can perform the test by yourself. Feel free to ask questions along the way.

**Activity:** Given permission, go through test together. Encourage them to ask questions along the way.

1. Do it together,
2. Respondent can test by himself/herself (asking questions as needed).

The Compartment Bag Test will be ready to read this time tomorrow. In the bag, there is food for microorganisms to grow and reproduce. There is also a special dye that will turn blue if there is *E. coli* present. *E. coli* is a bacteria and an indicator that the water may/may not be safe for drinking. If no *E. coli* is present, the water in the test will look like it does now and not change color. Tomorrow I will come back and we will look at the results of your test from your water sample together.

What time tomorrow is best for me to come back?

I also want to mention that there are some limitations of the Compartment Bag Test.

1. This test only tests for *E. coli*, not other microorganisms or chemicals.
2. The result from this test is only based on one day. Water quality changes so if you get a certain result on one day does not mean that it is permanent/constant.

**General Feelings/Reactions – Compartment Bag Test**

13. What do you think about the test? What are your reactions?

15. How safe do you think your water is? [Verbally read the scale]

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Very Unsafe</td>
<td>Unsafe</td>
<td>Neutral</td>
<td>Safe</td>
<td>Very Safe</td>
</tr>
</tbody>
</table>

16. What do you think are the advantages/disadvantages of using this test?

17. When might you use the test? What scenarios? (Seasonal, social, life stage)
18. Why would you want to use this test? Are there things (incentives) that would make you want to use it? (What might be your incentives to use this test?)

Usability
19. Do you think you would use this test? Y / N If Y, when?

20. Would you be able to use this by yourself without me? Y / N

21. Could you teach others how to use this? Y / N

22. If there is a step in the use of this test that is confusing/difficult – what is it? How can it be improved?

23. How can I improve my teaching others on how to use the Compartment Bag Test?
Day TWO Interview

[Show results, read results together and pamphlet on options of improving water safety.]

REACTION TO RESULTS

1. What is your reaction from seeing the results?

2. How much do you like the test? [Verbally read the scale]

   1   2   3   4   5
   Dislike a lot Dislike Neutral Like Like a lot

3. How useful did you find the test? [Verbally read the scale]

   1   2   3   4   5
   Not Very Useful Not Useful Neutral Useful Very Useful

4. Has this bag test given you information you did not have before?

5. Now that you have seen the test, do you think you would use it, and if so when?

6. Please describe what you see and how you think it relates to your household water safety.

7. How safe do you think your water is? [Verbally read the scale]

   1   2   3   4   5
   Very Unsafe Unsafe Neutral Safe Very Safe

8. The Compartment Bag Test gave you another piece of information about the quality of your own water. Now that you know your water is (result) today, would you change your current practice? If so, how? And why?

9. Would you recommend using this to your neighbor? Why or why not?
REFERENCES


Health Organization.


