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Sarah P. Turner Date

Field Methods for Determining *E. coli* Concentrations in Environmental Samples: A Systematic Review

By

Sarah Turner

Master in Public Health

Hubert Department of Global Health

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Amy E. Kirby, PhD

Thesis advisor

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Sarah Turner

Bachelor of Science in Animal Science

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Thesis Advisor: Amy E. Kirby, PhD

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Abstract

Field Methods for Determining *E. coli* Concentrations in Environmental Samples

A Systematic Review

By Sarah Turner

It is critical to utilize environmental sampling and monitoring to detect contamination in different matrices such as soil, water, and produce to assess risk. While methods for determining microbiological contamination have improved dramatically over the last few decades, they can be difficult to execute in low-resource settings due to varying levels of infrastructure, human resources, and cost. Improved detection methods are not always considered feasible for low-resource settings but methods appropriate for these settings do exist and should be implemented as the results can indicate whether or not harmful exposure to pathogens is likely. This paper is intended to encourage increased uptake of environmental microbiological sampling in the field through a systematic review of the simple field methods for detecting and enumerating *Escherichia coli (E. coli)* as an indicator of fecal contamination.

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Chapter 1: Introduction

Worldwide, 748 million people lack access to improved drinking water and 2.5 billion lack access to improved sanitation, leaving them vulnerable to water, sanitation, and hygiene (WASH) related diseases (WHO, 2015). The lack of WASH infrastructure increases the risk of exposure to dangerous pathogens and the subsequent disease burden hinders economic development (Institute of Medicine, 2009). Estimates have shown that nearly half the population in low-income countries have infections or diseases related to WASH infrastructure (Bartram et. al, 2005). These settings are commonly hindered by other challenges as well such as lack of electricity, trained personnel, and funding, which makes routine monitoring of microbial risk challenging.

Due to these challenges, techniques used in laboratories in high-income countries to assess fecal contamination levels cannot always be implemented in the settings where they are most needed. Therefore, field methods to assess basic levels of contamination should be incorporated into a regular monitoring plan in low-resource settings as this would be better than no testing at all. Building infrastructure is time-consuming as well as costly and standards cannot often be raised to enable the use of advanced monitoring practices. Instead, environmental sampling and field methods can be used to assess risk and guide the design of interventions to reduce pathogen exposure within a population.

Exposure to pathogens, such as *Escherichia coli* (*E. coli*), in low-income countries or settings can occur on a daily basis as water, hands, and food, for example, can be contaminated with fecal matter. Children are especially vulnerable to diarrheal diseases from these exposures and WASH-associated diseases are responsible for 20% of deaths in children under 14 years of age (Bartram et. al, 2005). Infections can become chronic, leading to long term effects on their

health. These chronic illnesses and deaths mean loss of production and the chance for human and economic development in a society. It is increasingly important, therefore, to routinely monitor the bacteria load in water, sand, soil, and sludge as well as on surfaces, hands, and other fomites. This review is meant to aid practitioners in choosing the right field method for their setting and matrix.

1.1 Background and Rationale

Urbanization. The world is becoming increasingly more urban. In 2014, approximately 54% of the global population was estimated to live in cities. Furthermore, 651 million of these urban residents live in slums or slum conditions and, as a result, the number of slums worldwide is increasing as well (WHO, 2015). With urban and slum living comes increased risk of exposure to pathogens due to challenges with WASH infrastructure and crowded living. Slums, which are defined as dwellings with lack of access to improved water, sanitation, sufficient space, durability and security, need monitoring of food, water, hands, and the other environmental risks that cause disease (U.N., 2008).

Low-income countries are generally thought of as places where these slums commonly exist. They are defined by The World Bank as those that have a gross national income (GNI) of $1,045 or less (World Bank, 2015). Despite the World Bank definition, there is no universally agreed upon definition of what constitutes a low-income country in terms of other metrics. In this review, however, the term low-income country is referencing countries that met the definition put forth by the UN as a Least Developed Country (LDC). An LDC is a country with extreme poverty, weak human resources in terms of health, nutrition, education, and literacy, and economic vulnerability (United Nations, 1971). Many LDCs also have poor infrastructure that leads to decreased access to health facilities and further isolation of pockets of the population.

Low-resource settings, which are more often found in LDCs, do not always have standard guidance protocols on the best way to monitor for microbial contamination (Crocker and Bartram, 2014). Low-resource settings consist of one or all of the following characteristics: no or inconsistent electricity, no advanced lab equipment, lack of or shortage of trained personnel, limited or no suppliers of lab equipment, and lack of infrastructure for waste disposal. Another systematic review conducted solely on testing methods for drinking water defined low resource as any setting without a lab, but with a clean work space or near the water source (Bain, et. al, 2012).

High-resource settings, in this same review, were defined as places with a modern laboratory accessible within 24 hours. The lab should have reliable electricity, a vacuum, fume hood, distilled water, and cold supply chain (Bain, et. al, 2012). This definition is also useful for this review. When reviewing articles and the methodology utilized, it was noted what type of lab the testing was done in or if field based monitoring techniques were used that could be implemented in other low-resource settings.

Diseases linked to WASH. The WASH-associated disease burden in slums and rural areas in low-income countries can be disproportionately high compared to high-income settings and is particularly high in children. WASH-associated diseases are caused by bacteria, such as *E. coli* or *Campylobacter,* viruses such as Norovirus, or parasites such as Giardiasis (CDC, 2012). Worldwide in 2010, approximately 800,000 children died from WASH-associated diarrheal disease (CDC, 2013). Contaminated food or water, and poor sanitation are major sources of exposure to enteric microbes. The subsequent disease outcomes are more severe in low- resource settings due to malnutrition and lack of access to treatment (Ashbolt, 2004). The diseases that are

commonly seen in children and are associated with slum living include asthma, malaria, and malnutrition (WHO, 2009).

Stunting, a common result of malnutrition or frequent diarrheal infections, currently affects approximately 178 million children under the age of 5 worldwide (Black et. al, 2008). It is defined as being 2 or 3 Z scores below normal height-for-age, which can lead to a lifetime of related co-morbidities and increased risk of death (WHO, 2015). Stunting has been hypothesized to be caused by environmental enteropathy (EE), which is a condition common in infants in low-income countries and has been shown to cause slow growth due to chronic inflammation of the intestines and reduced function of intestinal barriers (Ngure et. al, 2013). EE is thought to be caused, at least in part, by continued exposure to fecal pathogens and unsanitary living conditions (Humphrey, J., 2009). Fecal contamination represents a large portion of the risk for exposure to bacteria that may cause these illnesses.

Fecal Contamination. The most common exposure pathways to fecal contamination in unsanitary living conditions are thought to be the five F's: food, flies, fingers, field, and fluids. The ability to test the contamination level of each of these matrices is crucial and these fall under the more general matrices included in this review. They are gaining more attention in studies, compared to primarily water testing in the past. A study from 2010, for instance, found that mothers in Tanzania had up to 6,310 colony forming units (CFUs) of bacteria on their hands while preparing food (Pickering et. al, 2010). Another study in Mexico tested the toys and hands of children and found pathogenic bacteria and fecal coliforms (Martinez-Bastidas, et al., 2014). The importance of testing meat and other foods in low-resource settings cannot be understated. In the U.S., foodborne illness is estimated to affect 76 million people per year. In settings where

refrigeration is less common and accessible, meat and other perishables represent significant risk for pathogen exposure.

While not always the main exposure pathway, water is the matrix most often tested for contamination. Due to the known hazards associated with drinking contaminated water, the World Health Organization (WHO) developed guidelines for ensuring safe drinking water in 2008. They were adapted from the Hazard Analysis and Critical Control Point (HACCP) system used for food safety (Crocker and Bartram, 2014). This Water Safety Plan (WSP) has three components. First, an assessment of a water system is recommended to determine points of potential hazard. Second, control measures should be realized in order to reduce hazards. Finally, regular monitoring should take place to assess the systems function and management plans for regular and incident conditions should be in place (Köster, 2008). As is well documented, failure to monitor and ensure safe drinking water for a community is a public health risk. But despite this knowledge, it is unclear if low-resource settings could comply with a WSP (Cocker and Bartram, 2014).

This water safety plan, as it is described, seems more applicable to more developed settings with a piped water system in place. Ground and surface water sources, however, still need to be protected as they are a common source of drinking water in low-resource settings. Non-potable water, soil, produce, and other matrices should be included in the plan and monitored as they also represent exposure pathways. The promotion and implementation of easy field techniques would be an integral component of a WSP in low-resource settings for routine risk assessment as well as outbreak investigations (Bain et. al, 2012).

The WHO further recommends good personal and domestic sanitation practices to try to manage diarrheal risk that comes from the five F's at the household level, as they can be highly

contaminated (Gil et. al, 2014). Improving practices is difficult, however, and involves permanent behavior change. An important step towards achieving behavior change is demonstrating and quantifying risk in order to raise awareness and develop applicable interventions (Gil et. al, 2014).

General Approach to Detection. Currently, PCR, which is based on the detection of RNA or DNA, is the method that is easiest to adapt to new target organisms for testing. PCR was developed in 1983 and has been very important in diagnostics and research. It is highly adaptable and can detect viruses, bacteria, or unknown pathogens in a stool specimen with high levels of sensitivity and specificity. There are many reasons, however, why a PCR would not be appropriate or available for use in low-resource settings. A PCR machine ranges from $250 USD for a used machine to over $15,000 for a new one, requires electricity, cannot tolerate high humidity or heat, requires well-trained personnel to operate it, and requires a clean workspace.

Field methods can be used instead of a PCR machine to quantify risk in low-resource settings. They rely on the detection of indicator bacteria to demonstrate fecal contamination. Fecal indicators are most commonly used to indicate the possible presence of bacteria, viruses, and protozoans. Generally, there are three recognized groups of indicators: general microbial indicators, fecal indicators, and index and model organisms. The WHO outlined seven criteria of fecal bacteria that they deemed essential for use in water quality testing. The indicator should be absent from unpolluted water, easy to identify and enumerate, should be present in larger number than the pathogens, should respond to treatment in a similar way to the pathogens of interest, can be identified by an inexpensive test (so many samples can be done), should not be pathogenic and, finally, should not multiply in the environment (WHO, 2002).

Two of the most common fecal indicators used are total coliforms and *E. coli*. Generally, total coliforms are bacteria found in the environment, usually in soil or vegetation, and they are mostly harmless. Total coliforms are rod-shaped, Gram-negative bacteria that ferment lactose at 35-37 °C with the production of carbon dioxide and acid (Köster, 2008). Fecal coliforms are a subgroup of total coliforms and exist in the intestines of people and animals. *E. coli* is a subset of fecal coliforms found only in the intestines of people and warm-blooded animals (EPA, 2013). Since *E. coli* is a species of fecal coliform bacteria that is specific to feces from humans and other warm blooded animals, it has become the most common indicator of health risk (E.P.A, 2012). Furthermore, *E. coli* is the most suitable indicator due to its thermotolerant behavior, which means that, like other fecal coliforms, it ferments lactose at 37°C and 44°C. (Köster, 2008). This allows for its distinction from other coliforms that may not be specific to fecal contamination.

E. coli can be used to infer if pathogens may be present. Normal water distribution systems, however, do not support the presence of these bacteria, so their existence indicates a breach in normal function (Köster, 2008). The WHO mandated in their drinking water guidelines that no *E. coli* should be detected in 100 mLs (Bain, et. al, 2012). This standard can also be applied to other matric (es) when their *E. coli* levels are being tested, if a water wash is used to recover the bacteria (Bain, et. al., 2012).

Bacillus coli (B. coli), as *E. coli* was first known, was discovered in 1885 by Theodor Escherich who was a German pediatrician. He noticed that a large number of babies were dying of diarrhea and, as a student of the germ theory, believed he could find the organism responsible (Gallup, 2014). He discovered the *Bacillus communis coli* when he isolated it from breast-fed infants. *Escherichia coli*, as it came to be called after Escherich's death, is present in

concentrations of approximately 10^9 in mammal feces and, as mentioned above, does not multiply significantly in the environment (Edberg et. al, 2000).

Due to its presence in feces and behavior in the environment, it was chosen as the biological indicator for water treatment efficacy in the late 19th century. Shortly after, testing methods for total coliforms and fecal coliforms became more popular due to a lack of an efficient method to test for *E. coli* directly. When Defined Substrate Technology (DST) emerged in the late 1980s, *E. coli* once again became the most commonly used indicator of fecal contamination (Edberg et. al, 2000). It is known as a 'model organism' due to its experimental advantages (Gallup, 2014). Field testing methods rely on such 'indicator organisms' to determine if a matrix has been compromised by a recent fecal contamination event that could lead to fecal-oral exposure and subsequent illness.

There are some advantages to field methods compared to lab methods. The samples are fresh and will not be subjected to the changes that can occur during transport or potential contamination from a storage container. It is also easy to re-sample if needed. It should be clarified, however, that lab testing is preferable to field testing as the results will be more valid and all other variables can be more easily controlled, but field testing is better than not testing at all. Therefore, these field testing methods can and should be utilized more often to test for *E. coli* in a variety of matrix (ces).(WHO, 1996).

There are three general approaches to detecting bacteria using field methods. The first is the plate enumeration method, which allows for colonies to be counted and is the most accurate. The second is the most probable number (MPN) approach, which estimates the concentration of viable organisms in a sample. Serial dilutions of the sample are created in order to assess the presence or absence of bacteria in multiple slices of the sample. MPN is also useful when plate

count enumeration methods are not appropriate due to particulate matter in the samples (Sutton, S.). The third approach is the selective, differential detection approach, which allows for the detection of lactose fermenting enzymes, indicating the presence of coliforms and *E. coli*. This last method is specific but expensive.

The media that is used to grow and detect the bacteria through these approaches can be selective or differential. Selective media allows for the growth of certain types of organisms and inhibits the growth of others. Differential media is used to distinguish between organisms or groups of organisms that are closely related. Dyes or chemicals in the media will cause growth or changes in the organism that allows for differentiation (Amrita,n.d.).

1.2 Problem Statement

Due to the growing number of number of people living in urban settings, challenges with WASH infrastructure, and risk of WASH-related illnesses from bacteria exposure, it is imperative to conduct tests for microbial contamination in low-resource settings. The risk of exposure to potentially harmful bacteria is high in these settings and field testing is a necessary step towards monitoring the risks and improving WASH standards.

Field testing and lab testing usually work in conjunction with each other in high-resource settings. In low-resource settings, field testing is usually the only option and is often overlooked by practitioners. Field testing methods can still be executed by setting up a makeshift laboratory in a school classroom or the health clinic, for example. The samples need to be incubated after collection in order for the bacteria to grow and be detected. Incubators are portable, however, and several samples can be incubated simultaneously. Incubators require electricity, but are still much simpler to use than advanced detection methods that utilize PCR. If there is no electricity

or no incubator, samples can be incubated using body heat. The challenges to conducting microbial testing in low-resource settings are legitimate but they do not have prevent testing altogether.

1.3 Objectives and Rational

This systematic review was conducted to determine which tests work best in field settings. Field methods used to detect and enumerate *E. coli* in low-resource settings were identified. These methods were evaluated for field use capability with the following criteria in mind: equipment, training, cost, and matrices they have been used for previously. Finally, information regarding sensitivity and specificity was collected from the manufacturers and published literature. The testing methods that are highlighted in this review are Colilert®, AquaTest, Petrifilm, Delagua, the Compartment Bag Best, and the Hydrogen Sulfide Test.

Other similar systematic reviews have been done regarding best testing methods for low-resource settings but have focused solely on drinking water. This review will also analyze these methods in terms of testing other matrices mentioned above that are sources of exposure such as soil, hands, and produce. The relative costs, efficacy, and level of difficulty of each method will also be evaluated when possible.

This review has six primary research questions.

1. What field methods are available for *E. coli* detection and enumeration?
2. What are the equipment needs for each method?
3. What are the training requirements for each method?
4. What are the costs associated with each method?
5. What types of samples have been tested with these methods?
6. How does the sensitivity and specificity of these techniques compare to lab-based detection methods (membrane filtration, PCR)?

1.4 Significance statement

This review will aid field practitioners in choosing an appropriate method for testing for microbiological contamination using *E. coli* as an indicator for other fecal contaminants in low-resource settings. There is existing literature available to aid in this decision making process in the context of water. Other matrices that are relevant exposure pathways will be discussed here and will encourage the increased integration of environmental microbiology into future studies.

Chapter 2: Methods

This review was conducted by systematically searching PubMed, Environment Complete, World of Science, and Environmental Science and Pollution Management (ESPM). The databases were revisited periodically to screen for new publications. Gray literature was also collected from the internet on guidelines, standard operating procedures, and manufacturer's recommendations for use for each testing method. A meta analysis was not conducted.

All searches conducted in the aforementioned databases included ("*Echerichia coli*" OR "*E. coli*") and combinations of search terms related to testing methods ("colilert", "Del agua", "AquaTest", "portable membrane filtration", "membrane filtration" and "petrifilm") and matrices ("water", "soil", "hands", "hand rinse samples", "sludge", "fecal sludge", "sand", "food", "sewage", "surfaces" and "produce") as well as the setting ("low-resource settings", "low-income settings", and "developing countries".)

Citations were exported to Endnote X7 for review. Titles and abstracts were reviewed to screen for potentially relevant studies. Full text articles were obtained for all studies that were deemed relevant after reviewing their title and abstract. The full texts were further evaluated

within the scope of inclusion criteria and studies that were still applicable were put into a new database in Excel for analysis. The inclusion criteria for this review included:

- Studies or reviews that utilized low cost, easy to use field methods to assess microbiological contamination using *E. coli* as the indicator bacteria in matrices including, but not limited to, water, soil, sludge, fecal sludge, sand, hands, hand rinse samples, food, produce, sewage, and surfaces.
- Studies conducted in or reviews focusing on low-resource settings or low-income countries;
- Reviews or studies conducted between 1995 and 2014;
- Published in English; and
- Full text available through the Emory Library or other catalog system.

Excluded criteria included:

- Reviews or studies that utilized analysis methods in a lab that could not be used in low-resource settings (i.e. PCR, EIA), even if the sample was collected in a low-resource setting;
- Reviews or studies not published in English;
- Reviews or studies published before 1995; and
- Reviews or studies in which the target organism was not *E. coli.*

E. coli is the main microbiological contaminant of interest because it is a universally accepted indicator of fecal contamination. Other pathogens are less understood and/or are not specific to fecal contamination (Köster, 2008).. Therefore, papers that detected *E. coli* as well as

other pathogens were included, but those that were specifically testing for other pathogens, excluding *E. coli* completely, using these methods were not included in the review

In an effort to stay current, included reviews and studies were restricted to the last twenty years. It was decided that a broad search could be used since the published literature is not very extensive and some of the technologies have not been written about exhaustively. Data extracted from each article included reference, year, testing method, matrix (ces) tested, sensitivity and specificity (if mentioned), cost per unit, and start up cost. The studies that were excluded based on their applicability to low-resource settings were done subjectively, but with the criteria of electricity, training, and financial needs in mind.

This review did not require submission to the Emory Internal Review Board.

Chapter 3: Results

The results of this systematic review were compiled in this chapter and organized by method. In total, 137 relevant articles were identified. Each method is discussed separately and each matrix is discussed per method where applicable. The cost and equipment needs for each method are outlined as well.

3.1 Petrifilm

A. History of Petrifilm

The 3M-food safety company developed Petrifilm in 1984. Since its initial launch, Petrifilm has become known as a fast, simple, easy test for microbial contamination. Petrifilm is a ready to use, dehydrated version of the conventional Petri Dish agar plate. They are a paper-thin film and can be used immediately upon opening the packet. They can also be stacked during

incubation, and since they are so thin, little space is required. They should be kept refrigerated before opening at an approximate temp of ≤8˚C (≤46˚F) but brought to room temperature before use. (Carolina, 2015). Other advantages include easy enumeration capability, easy to prepare, and small volume requirement. The small volume requirement can also be a disadvantage, however, because many dilutions may be required to enumerate bacteria if the expected contamination level is uncertain. There are 14 different Petrifilm tests available, each targeting a different bacterial species or groups of bacteria.

The specific type of Petrifilm of interest in this review is the *E. coli*/Coliform (EC) count plate. It contains Violet Red Bile (VRB) nutrients, a cold-water soluble gelling agent, an indicator that allows for colony enumeration, and an indicator of glucuronidase activity (3M, n.d.). Most *E. coli* produce beta-glucuronidase, which produces a blue color that can be counted as a colony. *E. coli* and coliforms ferment lactose and that process produces a gas that is trapped by the film. If coliforms and *E. coli* are present, the test will produce gas but an *E. coli* positive test can be differentiated as blue colonies with gas (3M, n.d.).

The plates have a grid on them so the colonies of bacteria produced can be easily counted. The *E. coli* that can be enumerated from one Petrifilm ranges from no growth to 200 cfu/ml. Only 1 mL of sample can be put on the film so if the results are 200 cfu/ml or higher, then the dilution needs to be adjusted. There is a 5 ml plate available for coliforms, but it does not allow for the discrimination of *E. coli.* A significant disadvantage of Petrifilm is the high limit of detection, 1 cfu/ml, which is equal to 100 cfu/100 ml and well above the WHO standard. Furthermore, it requires arguably more skill for use compared to some of the other methods but the plates provide a permanent record of the results (Metcalf, et. al, 2003).

B. Petrifilm with different matrices

Petrifilm AC (aerobic count), which is traditionally used for detecting yeasts and molds in food, has been used previously to try to enumerate coliforms in water but is not designed to handle the growth of bacteria found in water (Schraft and Watterworth, 2005). The Petrifilm EC plates, however, are becoming more commonly used in water testing and counts comparable to culture methods are expected. One study from South Africa tested dishwater samples using Petrifilm EC and found *E. coli* in 18 (78%) of the water samples (Mosupye and von Holy, 1999). Another study from Brazil found that Petrifilm was a good alternative to the MPN method for recovering *E. coli* from water, but only if colonies were expected at a load of 20/100 mls or higher (Beloti, et. al, 2003).

Petrifilm has been used to test for bacteria on surfaces and fomites. In Peru, a team of researchers tested children's meals, drinking water, kitchen utensils, and caregivers and children's hands for *E. coli* using Petrifilm. Hands were swabbed and the swab plated directly on the Petrifilm or hands were massaged in a bag of diluent. A 1 ml sample of the mixture was then placed on the film. After the Petrifilm was incubated, the presence of colonies would demonstrate the risk of illness from hands and everything they touch (Gil, et. al, 2014). This study is especially important in demonstrating the amount of risk that can be ubiquitous in the household. The most contaminated parts of the house were the kitchen cloths, and drinking water. The drinking water was commonly boiled but then stored in contaminated containers before consumption. The caregivers represented another high risk of exposure as 17 out of 19 (76%) had total coliforms on their hands.

Petrifilm was originally designed for use in food safety. As such, Petrifilm is a commonly used testing method for assessing microbiological contamination risk in food and there is more

literature evaluating its use in food than other matrices. Generally, the literature supports its use in the evaluation of poultry and beef carcasses as well as other meat products. It should be clarified, however, that the majority of the literature found on Petrifilm summarized studies that were done in lab settings or in processing plants rather than in the field. The ability of Petrifilm to recover *E. coli* in various matrices has been demonstrated, and this is an important point to establish before discussing its use in the field.

In high-resource settings, Petrifilm is commonly used in slaughter and processing plants. The procedure for testing in these settings is as follows: first, the food sample or swab of the food sample is collected in a whirl-pak bag, stomacher bag, dilution bottle, or other sterile container (3M, n.d.). Then, a 1:10 dilution or greater is created using Butterfield's phosphate buffer (IDF phosphate buffer, 0.0425 g/L of KH2PO4 adjusted to pH 7.2), 0.1% peptone water, peptone salt diluent (ISO method 6887), buffered peptone water (ISO method 6887-1), saline solution (0.85-0.90%), bisulfite-free letheen broth, or distilled water. Finally, the solution is blended into fully liquid form and 1 mL is dropped on the plate.

This could be done in low-resource setting if the procedure could be simplified, but still may be beyond the skill of local personnel. Whirl-pak bags or some other container for sample collection would be available in low resource settings, although the sterility may be hard to maintain. The recommended liquids for dilution may be hard to obtain as well. Distilled water or peptone would likely have to be brought into the field for testing. One study from the U.S., however, found, that direct surface swabs are an equally sensitive method for *E. coli* recovery, which would simplify the process as blending is not required (Calicchia, et al.,1997). Unfortunately, Petrifilm also has a high lower limit of detection, so a pre-enrichment step, using

a lactose broth, would also be required if low amounts of *E. coli* are expected (Beloti, et. al, 2003).

As mentioned, the literature supports the use of Petrifilm in testing meat products. Two studies, out of the U.S., concluded that Petrifilm is a reliable, easy method for recovery of *E. coli* from beef samples, whether they were frozen, fresh, or ground (Restaino and Lyon, 1997; Linton, Eisel, and Muriana, 1997). Beef carcasses evaluated for safety using Petrifilm found the Petrifilm results were significantly related to the LST-MUG test ($P < 0.05$) and within the same 95% confidence interval in all but one sample.

Poultry products have also been tested for contamination using Petrifilm. A study from Canada detected *E. coli* on turkey carcasses using 10 ml sample of muscle and successive dilutions up to 10^3 (Mallia, J.G., et al., 2000). Another study out of the U.S. that tested poultry products concluded that the mean log counts of detected *E. coli* were not significantly different between the Petrifilm method and MPN methods (Gangar, V., et al., 1999).

Its efficacy in evaluating contamination of fish and other seafood is less documented. A group out of the UK tried to use Petrifilm as an alternative to the MPN method to enumerate *E. coli* in shellfish. They found it to be an inadequate replacement (Ogden, I.D., et al., 1998). Another group in Argentina tried to recover *E. coli* from fish but found that other plating methods were more useful than Petrifilm (Sanches Muratori, et al., 2000).

Petrifilm was able to detect *E. coli* in cheese and was deemed an easy method with high confidence (Amaro-Berzunza, S., et al., 2001). In another study from Brazil, *E. coli* was detected using Petrifilm in 10% of samples of raw milk and cheese with counts of 1–2.18 log CFU/mL

(Costa Sobrinho Pde, S., et al., 2012). The methods described in these studies, however, were performed in laboratories and not the field.

Petrifilm for the evaluation of contamination in produce has been demonstrated. One study out of Denmark evaluated lettuce for the presence of *E. coli* using Petrifilm. The lettuce was grown in a field that was fertilized with 'animal slurry.' Of the lettuce samples tested, approximately 50% were found to contain *E. coli* and, in 19% of these, the count exceeded 2 log CFU/g (Jensen, et. al, 2013). Another study done in the U.S. found limits of detection of 1.39, 1.60, and 1.76 log CFU/g for radish, arugula, and carrots respectively using Petrifilm. This study did use Butterfield's phosphate dilution (BPD) to make the solution for testing, however, so this would have to be brought to the field if possible.

Soil has been tested using Petrifilm. Soil can be rinsed and that water becomes the sample. The two studies mentioned above in relation to produce also successfully recovered *E. coli* from soil using Petrifilm (Jensen, et. al, 2013 and Natvig, et. al, 2002). In the study by Jensen et. al, the soil that was fertilized with animal slurry was found to be positive for *E. coli* using Petrifilm and, in the study out of the U.S., Natvig et. al found that the low limit of detection for *E. coli* in soil using Petrifilm is 1.29 log CFU/g (Natvig, et. al, 2002).

Lastly, feces can be tested directly using Petrifilm. In Australia, fecal samples were tested from beef cows at slaughter. *E. coli* was detected at 5 x 10(5) and 2.5 x 10(5) CFU g(-1) for lot- and grass-fed cattle, respectively (Fegan, et. al, 2004). Neither of these studies took place in low resource settings, however, and the processing had significant lab components. Regardless, the result do demonstrate that Petrifilm can be used to evaluate contamination in feces if the methods can be appropriately simplified for field use.

C. When to choose Petrifilm

Petrifilm should be used more frequently in low-resource settings. It is recommended, however, that, in the field, a presence/absence test should be used first, and, if positive, the Petrifilm used second for enumeration. If that test is positive, it is can be assumed that a high level of *E. coli* is present. If it is negative, then a low level of *E. coli* is present and 10 10ml samples should be tested using presence/absence MPN (Harvey, 2007).

Sensitivity. Petrifilm has also been found to have low sensitivity, 39.5 to 52.5% but high specificity, 78.8 to 90.9% in water (Hörman, A. and M.-L. Hänninen,2006). In general, the mean counts of *E. coli* recovered in water were not significantly different than food, which is positive since the product was originally designed for use in food. (Vail, et. al, 2003).

Cost. Petrifilm costs about $1.30 per unit, which is relatively lost cost for a quantitative test (Bain et. al, 2012). When considering Petrifilm for use in water testing instead of Colilert®, Petrifilm is advantageous in terms of equipment costs. They both require an incubator but Petrifilm does not require the sealer that the Colilert® does, which can cost approximately $4,000 USD. Petrifilm is not as sensitive and may require more dilutions, however, which would increase the number of tests needed. Interpreting tests correctly also requires slightly higher skill level or training, which means more capital cost.

Waste. Petrifilm does not generate a lot of waste. The plates are small and can be easily disposed of in whatever matter is appropriate for the setting. The sample bags will also have to be discarded after use. The bacteria is not contained though so the handler should be aware of this factor.

3.2 Colilert® Quanti-Tray®

(IDEXX, n.d).

A. History of Colilert® method

The Colilert®-18 Quanti-Tray® is a most probable number (MPN) method of enumerating bacteria developed by IDEXX laboratories in Maine, USA. The Quanti-Tray® 2000 has large and small wells and, when the presence of bacteria is detected in any of them, a color change occurs. These are the positive wells and they can be used to statistically determine the likely density of bacteria (Aulenbach, 2010). Quanti-Tray®, containing only large wells, and Quanti-Tray®/2000, containing large and small wells, are the two most commonly used trays. The Quanti-Tray® has 51 wells that provides counts from 1-200/100 mL and the Quanti-Tray®/2000 provides counts from 1-2,419/100 mL. The Quanti-Tray®/2000 provides a huge advantage for water testing as it is based on the statistical model of a 15-tube serial dilution. This removes the need for multiple dilutions in the field and cuts down on time, equipment needs, and training. It should be mentioned, however, that in a lab setting, serial dilutions using Colilert® Quanti-Tray® may still provide the most accurate picture of contamination levels (Govender, et. al, 2011). The Quanti-Tray® method uses a medium, Colilert®-18, that contains two enzyme substrates that react with

total coliforms and *E. coli.* Colilert®-18 also suppresses the growth of other non-coliforms that could produce a false positive result (Aulenbach, 2010).

The technology behind this method is pretty straightforward. It utilizes defined substrate technology (DST) to detect fecal coliforms and *E. coli* simultaneously using Colilert®, which is an enzyme-specific liquid broth medium. In Colilert®, *ortho*-Nitrophenyl-β-galactoside (ONPG) is metabolized by coliforms and turns yellow and methylumbelliferyl-β-D-glucuronide (MUG) is metabolized by *E. coli* and will fluoresce blue under UV light (IDEXX, 2013). Colilert® can be used in presence/absence tests, Quantity-Tray® or Quantity-Tray 2000®, or in multiple-tube MPN methods. Colilert-18® is an newer, enhanced formula that can provide results six hours faster, at 18-hours rather than the 24-hours required for Colilert® (IDEXX, 2013). This method is the new International Organization for Standardization (ISO) standard for detecting fecal coliforms and *E. coli* in water (IDEXX, 2013).

B. Colilert® methods in Matrices

As the Colilert®-18 Quanti-Tray® has become the standard in water testing, the literature supports its utility. This review found 91 articles using 'Colilert®' paired with a number of other search terms in three databases and 48 articles met the inclusion criteria, of which, 39 were water-related. The Colilert®-18 Quanti-Tray® method was used for *E. coli* detection in sea water, drinking water, surface water, swimming areas, and recreational waters. This method does not require a lot of training to learn or hands-on time to process, but its use for water testing in the field has been questioned (Miller, 2013). Most of the studies reviewed below which tested water using Colilert® ultimately transported samples back to a lab or makeshift lab for incubation. There is some evidence, however, that it could truly be utilized in low-resource setting without a lab setting.

In a study comparing water testing methods, Colilert® produced results that were most consistent over the other methods, with the lowest coefficient of variation (Wohlsen, 2006). Furthermore, results obtained using Colilert® have been deemed more sensitive than results from membrane filtration (MF) and the method also requires less equipment, less training, and is faster. MF methods using mFC agar allow for the growth of organisms other than *E. coli* that can inhibit the growth of *E. coli*, which can alter the results (Wohlsen, 2008).

Stored and well water can be evaluated using Colilert-18® as well. In a household study from Ivory Coast evaluating the quality of stored water, the results showed levels of *E. coli* contamination detected by Colilert® and MF to be strongly correlated ($r^2 = 0.93$), indicating that Colilert® is an adequate substitute for MF methods in low-resource settings (Macy, 2005). In Dar es Salaam, water from vendors and shallow wells was tested and *E .coli* recovered using Colilert®. Only small volumes of water could be tested from the shallow wells and vendors in this study, but it was enough to indicate they may represent areas of high contamination (Metcalf, 2003).

The use of Colilert® in different types of water has been evaluated. One study from Taiwan was able to demonstrate in the field that Colilert-18® recovered equal or higher amounts of *E. coli* in freshwater samples than was recovered by MF (Chao, et. al, 2003). Furthermore, the false-positive rate of detection was 7.4% and the false negative rate of 3.5% for *E. coli* recovery in these samples (Chao, et. al, 2004). Whether or not Colilert® can effectively detect *E. coli* in seawater samples is less clear. Marine vibrios, such as *V. vulnificus,* if present in the sample can produce enough β-D-galactosidase to give a false positive result. Using distilled water to reduce the salinity of the sample has been effective in reducing some, but not all, interference (Sousa, et al., 2010).

This interference could be an important consideration in low-resource settings when multiple dilutions or confirmation testing may not be possible. Furthermore, Colilert-18® has been shown to be more effective than MF methods in recovering *E. coli* from chlorinated water, which has important implications for drinking water in low-resource settings. It has also been shown to better recover *E. coli* from chloraminated water, which may need to be tested in hospital settings to ensure water used for cleaning has not been compromised (Hallas, et. al, 2008). Finally, one study demonstrated that recovery of *E. coli* using Colilert® was not lessened within the range 33–39 ˚C, which may make ambient temperature incubation possible in low-resource settings in tropical or temperate climates (Matthews, 2014).

The benefit of using the Colilert® Quanti-Tray® method with food is less clear. Only one article found in this literature search made a definitive conclusion. A group of researchers in Japan and the U.S. made three dilutions of 10 ml food homogenate and injected them into a Colilert® medium for a presence/absence test. Four out of 27 samples that were found to be negative for *E. coli* using Colilert® were deemed false negatives when analyzed by other U.S. FDA methods. This paper mentioned that other studies had found similar amounts of false positives when using Colilert® in food testing, which was likely due to the β-glucuronidase in some foods, particularly fish and shellfish (Venkateswaran, K., et. al, 1996).

It is recommended, as a way to eliminate false positives caused by other auto fluorescent materials, to pre-treat the sample, by culturing it in a lactose broth, before *E. coli* testing (Venkateswaran, K., et. al, 1996). This extra step may be out of the scope of practice for field practitioners testing in low-resource settings, and as such, Colilert® Quanti-Tray® methods should be avoided for use in foods.

The data on the use of Colilert® for *E. coli* detection in the environment in the form of feces, fecal sludge, sand, and soil is lacking as well, especially in a field setting. One study conducted in the U.S. used Colilert® to recover *E. coli* in environmental sediments believed to be contaminated with animal feces. The objective of the study, however, was to see how long different strains of *E. coli* persisted in environment and so, although Colilert® was used, it is unclear whether it is the ideal method to use for sediment testing (Kiefer, L.A., et al.,2012). In New Zealand, Colilert® was also used to enumerate *E. coli* in feces and soil samples and it was found to be efficient at doing so, as long as the solid content was not too high (Muirhead, et. al, 2004). It was also found to be useful in recovering *E. coli* from waste activated sludge in Canada (Kramer and Liu, 2002). One study used Colilert® to test paper sludge that is used in agriculture, including fruit and vegetable production for *E. coli.* The authors were not completely convinced that *E. coli* could be recovered adequately enough with Colilert® to represent contamination levels since paper sludge has not often tested with this or any method (Beauchamp, 2006).

C. When to choose Colilert®

Sensitivity.

Table 1. Reported Sensitivity and Specificity of Colilert® methods in water testing

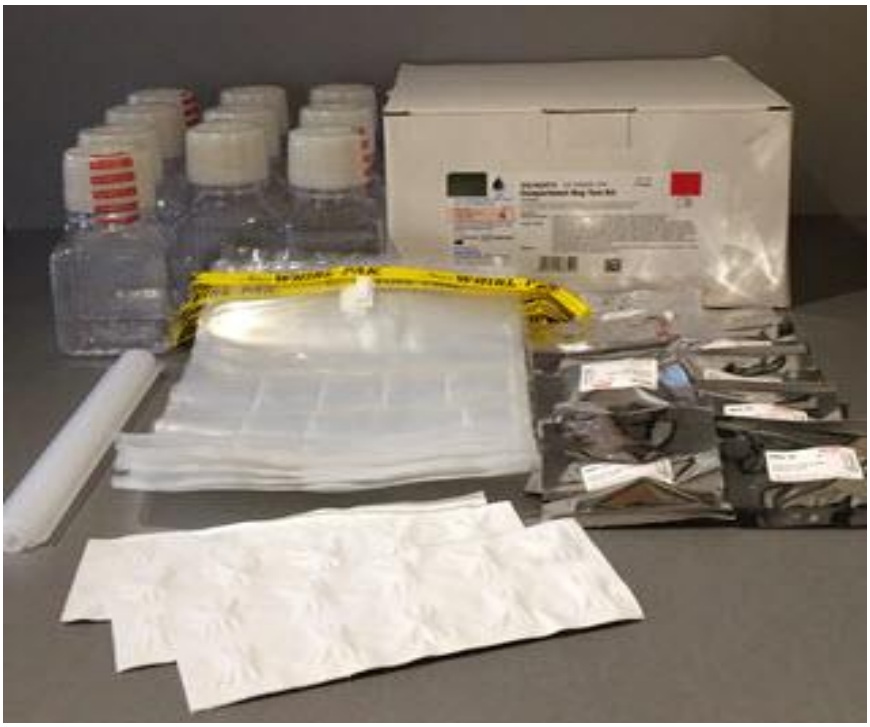
Author	Sensitivity (%)	Specificity (%)
Arnold, et. al	89	85
Brown, et. al	88	76
Maheux, A.F., et al.	93.2	94.4

The shelf life for Colilert® is approximately 12 months at room temperature; if used after that, its ability to suppress non-target organisms is diminished and the rate of false-positives increases (Landre, et. al, 1998)

Cost. Colilert-18® Quantity-Tray® methods offer the most advantages in water testing compared to other methods and other matrices. They are easy to learn, use, and the hands on time is short (Miller, 2013). The capital costs for these methods, however, can be very high comparatively so other methods could be considered or the Colilert® method adapted as will be outlined below. The equipment for conducting Colilert® tests costs approximately $6,000 as capital and about $5.50 per test, which consists of the IDEXX tray and Colilert® reagent powder (Miller, 2013). An incubator as well as a sealer would be included in the capital cost. A sealer is used to close the tray for incubation after the sample is mixed with Colilert® powder and poured into the tray. The sealer also requires a rubber insert to guide the tray through it during the sealing process, but this is included with the sealer. A sealer costs approximately $4,000 dollars, which is likely too much in most low-resource settings (Bain, et. al , 2012).

Waste. There is not much waste generated with Colilert®. The trays do have to be thrown away, but it is unlikely that there will be so many that the trash is cumbersome. Also, the potentially contaminated sample is contained so there is no added danger of exposure after testing. The whirl pak bags cannot be reused.

3.3 Compartment Bag Test



(Aquagenx, n.d.)

A. History of Compartment Bag Test

The compartment bag test (CBT) is an alternative MPN method, similar to Colilert® Quanti-Tray®. It is a product of Aquagenx, LLC, a social enterprise than was formed at University of North Carolina (UNC)'s Gillings School of Global Public Health. It was developed in 2012 and first sold commercially in 2013 (Aquagenx, 2015). It is a portable, self-contained water quality test that can be used at the household level to detect and enumerate *E. coli.* It does not require specialized equipment, training, or an incubator, if the ambient temperature is above 25 degrees Celsius. CBT significantly reduces costs associated with water testing such as refrigeration and transport to the lab (Aquagenx, 2015).

The CBT tests samples of 100 mLs of water, which is the volume recommended by the WHO for fecal indicator testing. It has 5 compartments which each hold a different volume of the sample once it is poured into the bag to simulate 5 different test tubes. The compartment bag

itself is sold as part of a larger kit for water testing and there are two different kits that can be purchased. CBT I has 10 compartment bags, 10 *E. coli* test buds, 10 100 ml plastic sample bottles, 30 chlorine tablets, and one seal clip. The CBT II has 50 compartment bags, 50 *E. coli* test buds, 50 100 ml whirl-pak Thio bags, and one seal clip (Aquagenx, 2015).

The CBT is conducted similarly to the Colilert® test. One hundred ml of water are collected into the bottle or whirl-pak bag, the test bud is added, and should be allowed to dissolve for 15 minutes, swirling occasionally, and then poured in the compartment bag. The bag should manipulated manually to ensure the fill lines for each compartment are met and then sealed with the provided clip (Aquanex, 2015).

The CBT works using a coliform medium containing X-Gluc as the basis for E. coli detection, compared to the MUG hydrolysis detecting method that Colilert® uses, which detect the same enzyme using different substrates. When a sample is positive for *E. coli*, therefore, it turns a blue/green color and stays yellow or yellow/brown when negative. The provided MPN chart allows for the *E. coli* colonies to be enumerated as well as classified on a scale from no risk to high risk or unsafe (Aquagenx, 2015).

B. CBT in matrices

There are some pros and cons to this method as well. It is a newer product, but so far, the results of its use show that it detects *E. coli* in water as reliably as the Colilert® method. Median values of *E. coli* recovered from Colilert® and CBT were compared using a two-sided t test and the difference was not significant with a p value of 0.45. It has not yet been tested for use in other matrices or in washes of hand or food samples.

C. When to choose CBT

The technology does have some disadvantages. It detects *E. coli* and, therefore, the presence of total coliforms as an indicator of possible pathogen presence goes undetected. Furthermore, it does require incubation if the ambient temperature is not at least 25°C and the recommended incubation times for relevant temps are: 35-44.5°C: incubate 20-24 hours; 30-35°C incubate 24-30 hours; and 25-30°C incubate 40-48 hours. To facilitate fieldwork, the manufacturer recommends a portable incubator or insulated container, if ambient temperature is too low.

Sensitivity. A study conducted out of Georgia State to evaluate the compartment bag found its sensitivity and specificity to be 94.9% and 96.6% respectively and the positive predictive (PPV) to be 99.6% and negative predictive values (NPV) to be 70% respectively. The false negative rate was found to be 5.1% (Miller, 2013).

Cost. The CBT I is sold as kits of 10 and the price ranges from $100 for 1-2 kits to $56 dollars/kit if 1751-2000 are purchased, which translates to $5.60 per test if the larger volume is purchased. The CBT II is sold in kits of 50 tests. Its price ranges from $468 for 1-2 kits to $262 for 400 or more, which, again referencing the higher volume, translates to $5.24 per test (Aquagenx, personal communication). The capital cost is not likely to be high, perhaps just the cost of an incubator and minimal staff training.

Waste. There is minimal waste generate by CBT. The kits come with chlorine tablets, which allow for safe disinfection of the sample before it is discarded and the whirl-pak bag and compartment bag themselves are the only materials that need to be disposed of, although this arguably could have high environmental impact if many tests are conducted.

3.4 Portable Membrane Filtration

Membrane filtration (MF) is a commonly used technique in labs and is approved for water quality monitoring in many countries. It allows for quantitative instead of semi-quantitative enumeration and can handle larger volumes of water. MF, as mentioned above, is performed by filtering a 100 mls of water through a funnel onto a sterile media. The media that are commonly used to detect total coliforms and *E. coli* simultaneously are MI agar, mColiBlue, lauryl sulfate broth, and MacConkey. MF, when done using MI agar medium, is highly specific and sensitive with low rates of false positives and negatives. The specificity of this media has been shown to be 95.7% (Miller, 2013).

Although it is a standard method for *E. coli* enumeration, membrane filtration has some challenges for use in field settings. The hands on time is only 15 minutes, but correctly interpreting the results requires skilled lab personnel (Miller, 2013). The variation in color of the colonies can make correct identification tricky without training. This technique also requires maintaining sterility for the medium and performing a positive and negative quality control test each time a new batch of MI agar is opened, all of which could be difficult in the field. While personnel could potentially be trained, the equipment costs can be up to $2,500 for the vacuum pump (Bain, et. al, 2012). Each media plate costs approximately $4.50 USD (Fisher Scientific, 2015).

Delagua, outlined below, developed kits to make MF portable and appropriate for field use in low-resource settings. While these kits are not always more affordable, they aim to be light-weight, easy to use, battery operated, and not generate much waste. They are potentially promising for use in the field, but there is a gap in data surrounding their use with anything other than water.

3.5 Delagua

(Delagua, n.d)

A. History of Delagua

The first Delagua water testing kit was developed in 1985 at the University of Surrey in partnership with OxFam. In 2008, the first improved kit was developed and launched based on customer feedback and the kits have been evolving ever since (DelAgua, n.d.). The kits are advertised as being useful for surveillance and monitoring water quality at the point of consumption, at the source, in water tanks, or at treatment plants. They are packaged in an impact resistant, waterproof, polypropylene case that has a hinged, lockable lid (Farma Mundi

Farmaceuticos, n.d.). They contain supplies sufficient for running 200 samples, as well as back up batteries and an electrical repair kit.

There are four kits that are applicable for water testing: the single incubator, the dual incubator, the bench top incubator, and the bacteriological kit number one. The kits, however, do not provide everything needed for field testing and it says that clearly on the website. They all require distilled water, a 1 litre measuring cylinder or beaker, clean cloths, methanol (approx. 1 ml per test), and access to a pressure cooker or portable autoclave (DelAgua, n.d.). The specific details for each of these four kits can be seen below in table 2.

B. Delagua with matrices

There were only two articles found, including the one mentioned above, that directly mentioned DelAgua kits. The second is simply an article written by an engineering group that was evaluating easy tests for bacteria detection. That publication did not draw any conclusions regarding the ease of Delagua in the field (Goodler, 2013). There is only a small amount of literature that demonstrates its ability to consistently recover *E. coli* in the field. One study from Peru tested fecal contamination of water using Delagua Kits but also tested contamination of hands, kitchen utensils, and food using Petrifilm (Gil, et. al, 2014). The DelAgua company, which was launched out of the University of Surrey in 2006, currently has a project in India that is using their product to test over 8,000 water sources. It is necessary to see data come out of that project that validate its utility in the field. Eventually the technique should tried for testing rinses of other matrices as well.

C. When to choose Delagua

Cost. There are some disadvantages to the DelAgua Kit in terms of cost. The kits range in cost from $2,000 to $5,000 USD, which is relatively a large capital cost (Bain, et. al, 2012). Furthermore, specialized training for interpreting the test would have to be included in the capital costs. For example, bacteria recovered using DelAgua kits in a study in Peru was enumerated by three separate lab microbiologists in order to obtain the most accurate count (Gil, et. al, 2014).

Waste. The test kits are not contained and, therefore, completed assays can present an exposure risk unless proper disposal precautions are taken. The method, in general, does not generate a lot of waste compared to the other methods, as only the filter and pad have to be discarded.

Table 2. DelAgua Tests

Test	**# of samples**	**Type of Test**	**Temperature**	**Power**	**Pore Size**
Single Incubator	16 test capacity	microbiological quality, turbidity, free chlorine, total chlorine, pH and temperature	44°C for FC	Internal battery, which can be re-charged from mains or a car battery	47mm 0.45µm pore size membrane filters
Dual Incubator	16 at 2 temps or 32 at 1 temp	TC, FC, turbidity, free or total chlorine, pH and temp	37°C for TC 44°C for FC	DC power adapter or external battery pack	0.45µm
Bench top incubator	16 test capacity	microbiological quality, turbidity, free chlorine, total chlorine, pH and temperature	44°C for FC	powered by connection to an external battery or to a cigarette lighter socket. The kit can still be taken	47mm 0.45µm pore size membrane filters

				into the field if needed (110V and 240 V)	
Bacteriological Kit number 1	200 samples	microbiological quality, turbidity, free chlorine, total chlorine, conductivity, Total Dissolved Solids (TDS), pH and temperature	44°C for FC	Internal battery is capable of up to 5 incubation cycles between recharges (12V)	not mentioned

3.6 Aquatest

A. History of Aquatest

Aquatest is a low cost, easy to use device that can detect *E. coli* utilizing the MPN technique. It was developed out of the University of Bristol in the UK in partnership with PATH international. The group, known as the Aquatest research consortium, is funded by the Bill and Melinda Gates foundation. In 2008, the consortium announced they were developing Aquatest and that it would be sensitive enough to detect 10 CFUs/100 ml of water (*Aiding the Fight Against Waterborne Diseases*, 2008). After that, the group partnered with Aquaya, a research and development group specializing in WASH technology, to evaluate Aquatest (Aquaya, 2014).

Currently, Aquatest is still being evaluated by commercial manufacturers who are interested in potentially licensing the intellectual property (IP). The IP is currently held by PATH and Aquatest will not be publicly available for purchase until a manufacturer invests in it (Ranjiv Khush, personal communication).

B. Aquatest with matrices

The turn-around time for water testing is similar to other tests. The incubation time needed is 24 hours and the analysis time is 5 minutes (Bain, et. al, 2012). Thus far, nothing is known about what other matrices, if any, can be tested using Aquatest.

C. When to use Aquatest

Cost. The Aquatest is slightly more expensive to other methods in terms of price per test but comparable in equipment cost. It costs about $4 per test and the capital cost for equipment is $100 for the incubator.

Flexibility. Its incubation temperature was tested for flexibility and it was able to recover *E. coli* without losing efficiency as long as the temperature range was maintained between 31-43 ˚C, instead of the exact range of 35°C ± 0.5 (Matthews, 2014). One significant limitation of Aquatest, however, is that it does not detect fecal coliforms.

3.7 Hydrogen Sulfide Method

A. History of H_2S method

The hydrogen sulfide (H_2S) test has not traditionally been used as an indicator for fecal contamination. It measures the presence or production of hydrogen sulfide, which encompasses total coliforms and *E. coli* but also includes many other bacteria, such as *Clostridium perfringens* and Salmonella. As such, this test, if used for *E. coli* detection can produce a lot of false positives (WHO, 2002). It can offer advantages, however, for low-resource settings that may make it applicable to water testing despite its specificity limitations.

The H_2S test can work in a couple of different ways. One study used test strips to detect *E. coli* in drinking water India. The strips have lead acetate on them, which reacts with hydrogen sulfide to produce a black precipitate in the form of a line on the strip (Tewari, et. al, 2003). Another study used Pathoscreen, a reagent for H_2S testing, mixed with 100 mL sample of water and incubated it for 24 hours in an MPN compartment bag. The organisms from the bag were serially diluted and plated on bacteria specific agars to determine the source of contamination (McMahan, 2012). A third method collected 30 mls of water, mixed it with the H_2S media, incubated it for 24-36 hours at room temperature. The samples were deemed positive if they turned black during that time frame (Khush, et. al, 2013)

B. H_2S method in other matrices

The H_2S test, as it discussed in the included studies above, is almost primarily used to test drinking water quality. Only one other matrix of interest in this review was found in relation to the H_2S test. The H_2S method was used to try to detect bacteria in sewage-contaminated water. The study was not done in the field and did have a lab component but the results are worth discussing. The authors compared H_2S method results to culture-based methods for contamination detection. They concluded that the H_2S method consistently detected fecal bacteria and there was a strong agreement between the two methods on which organisms were identified, which indicates better specificity than this method is thought to have (McMahan, 2011).

C. When to use H_2S test

Sensitivity. The H_2S test is very sensitive and can detect low amounts of sulfide. One study conducted in India found that the H_2S method was able to detect low-levels of *E. coli*

contamination in 96.4% of household water samples (CI: 89.9 to 99.3 at alpha 0.05) at the level of 10–99 E. coli CFUs/100 mL. At a higher level of *E. coli* , 100% of the samples tested positive for H_2S (95% CI 91.4, 100.0). Below 10 CFUs/100 mL, however, the test was not specific for *E. coli* contamination with only 53.3% (95% CI 48.7, 57.9) of H_2S positive tests actually identified as *E. coli* (Khush, et. al, 2013).

Another study out of Bangladesh compared the utility of the H_2S test to membrane filtration as an alternative for use in the field. They found that the H_2S test works better with samples that are highly contaminated (Gupta, et. al, 2008). Their results, however, indicated that the sensitivity and positive predictive value fell below 40% if *E. coli* levels were less than 1 CFU/100 mls. When the CFU/ml level was much higher in the sample, between 1000 and 9999, they found the sensitivity and specificity was 94% and 97% respectively and the positive and negative predictive values were 88% and 99% respectively (Gupta, et. al, 2008).

A group out of the UK did a systematic review on the accuracy of the H_2S method and their findings support this discrepancy as well. They found that as contamination levels were increased from 1 to 100 cfu/ml, the sensitivity increased, but the specificity decreased, and the same result could be expected as the volume of the sample increased. After reviewing their 19 included studies, the authors recommended using this method with caution and with expected bacteria levels and sample volume in mind (Yang, et. al, 2013).

Cost. It is low-cost and does not require a kit, lab equipment, sterile diluent, collection bottles or bags, or an aseptic technique (WHO, 2002). The different H_2S tests range in price from $0.60 to $2.40 per test and all but one have $0 equipment cost (Bain, et. al, 2012).

Waste. The amount of waste this method generates is unclear, although it is probably similar to the CBT method. It is also uncertain whether or not the sample is contained after testing is complete.

Some researchers have suggested that the H_2S test may work best in conjunction with another test in the field. It is useful as a presence/absence test, but then it may be necessary to identify and enumerate the bacteria. A study conducted out of MIT concluded that the H_2S test was best used with EasyGel.

3.8 EasyGel

EasyGel is an agar replacement medium that contains sugars linked to dyes that react with total coliforms and *E. coli.* It is a quantitative, enzyme substrate method (Chuang, 2011). Easygel comes in kits with a pre-treated petri dish and medium, which are sold together as one unit in sets of 10 units. If 1-9 sets are bought (1-90 tests), the cost is $21.25. If 9 or more are bought, the cost is $16.25, which means the individual tests range from $1.63 to $2.13 (Chuang, et. al, 2011)

In the study mentioned above, EasyGel reagent is mixed with 0.5 to 5 ml of water and pour into the pre-treated petri dish. Of the 83 samples run, there were 81 samples correctly identified as positive, 1% false positive samples, and 17% false negative samples. In low-resource settings without an incubator, the samples can be incubated at ambient temperature. This study did mention that, although the results of the H_2S-EasyGel combination of these techniques is very promising, it is currently not reasonable for monitoring water quality because

of the small sample size. They recommended optimizing the 20 ml H_2S test and testing on a larger scale (Chuang, 2011).

3.9 Challenges

As much as these methods are more feasible and applicable to low-resource settings, some challenges still exist for field implementation. Some of these challenges will likely apply to all settings and methods, but others must be weighed when choosing a method based on the setting of interest.

Quality control. The biggest factors to consider are: sample of interest, level of training, electricity, and cost. These can be individually or jointly problematic. In true low-resource settings, all four of these factors may be tricky for a monitoring plan. Supplies for quality control may be hard to get in country. However, equipment that is available and useful for quality control needs to be portable and easy to use. Furthermore, equipment will require maintenance and calibration. Re-calibrating when needed or testing a control sample along with regular samples may be seen as a significant burden on time or supplies.

A second quality control issue is that of sterility. The equipment needs to be clean and sterile. Devices such as an autoclave, which can be used to clean and reuse equipment, are often not portable. Therefore, one time use disposable equipment such as pipettes and swabs are useful for sample collection but can add extra financial burden (WHO, 1996). Running an autoclave will also require a highly trained person or extra money in the budget for training.

Waste Disposal. One time use equipment also adds to the burden of waste disposal and environmental cost. This is another thing to consider when choosing a method for field testing as some places may not have waste disposal infrastructure in place. The researchers will have to

dispose of the used materials in whichever way a setting allows and a method can be chosen with this in mind if disposal is expected to be problematic.

On top of environmental impact, the disposal needs to be safe. Some of the methods mentioned above allow for disinfection of the samples for safe, easy disposal, or include reusable materials. Whenever possible, efforts should be made to cut down on supplies that are needed per test that cannot be disposed of within the system the setting has in place; if not, reverse logistics will be needed to take waste back to the lab safely.

No electricity or incubator. Water samples, environmental swabs, or hand rinse samples were, in most of the reviewed literature, collected in the field and taken to some form of a laboratory for processing. This is not always feasible. Over one billion people in 50 countries do not have electricity and the majority of these people are in Africa and South Asia (World Bank, 2015). While this number is likely more representative of households, the settings in which these households exist may not have reliable electricity near by, if at all. In some of these places, like South Sudan, where less than 2% of people have access to electricity, field methods need to be able to perform successfully without it (World Bank, 2015).

Electricity is most important when testing for *E. coli* in terms of running the incubator. Petrifilm typically requires 24 hour incubation at 35°C for the colonies to grow. If no incubator is available, body incubation will work to obtain results. The sample is placed against the body for approximately 24 hours to grow the bacteria in the absence of an incubator. In the case of Petrifilm, a stack of up to 10 petrifilms can be stacked together and pressed between two pieces of cardboard secured with a rubber band. That stack can be placed against the body, using a body belt or tape, and incubated there. It is necessary to keep the sample against the body while

sleeping as well. Body incubation has been used with Colilert® as well, but with the P/A tubes, not the Quanti-Tray®.

One version of the Colilert® test, as mentioned above, can be used without access to an incubator. It is a presence/absence test that samples water for *E. coli* in tubes that contain a dry nutrient powder for the bacteria (Marks, n.d.). These tubes can be placed in a small sack or sock and kept close to the body to incubate during the day and slept on at night. A positive Colilert® test would turn yellow if coliforms are present, but a UV light would still be needed to detect *E. coli*. Portable UV lights that run on batteries can be utilized to read this test in settings without electricity.

One group of researchers at The Massachusetts Institute of Technology (MIT) has combined these two tests into one field testing the kit. The EC-Kit, as it is named, includes Petrifilm E.coli / Total Coliform Plates, WhirlPak bags, a Cooler Bag and Ice Pack, Colilert® 10 milliliter pre-dispensed tubes, an incubator belt, cardboard and rubber bands, 3.5 ml sterile plastic pipette, Blacklight and 4AA batteries, and laminated instructions. The group only recommends using these packs for testing of improved water sources, however, and believes that unimproved water sources should be assumed to be contaminated, which would save testing materials. Furthermore, they do not mention using their kits with other matrices but it is reasonable to assume to same 'incubation' techniques could be applied as long as the sample could be collected and processed.

The Environment and Public Health Organization (ENPHO), an NGO out of Nepal, developed a body belt incubator for use in the field as well. They field tested it in Nepal during a multiple indicator cluster survey about household water quality and have deemed it low-cost and reusable, although the exact price and whether or not it is for sale is not clear. Due to the success

in this study, UNICEF has expressed interest in its application in other countries. One of the most important qualities of the belt discovered during this study is that the samples should be unaffected by low ambient temperature as long as the belt is worn properly per instructions. Also, the results were compared to those samples that were grown in an electric incubator and no significant difference was found (ENPHO, 2014).

Another option for field processing of Petrifilm that is briefly mentioned in the literature is a water bath. Petrifilm EC plates were sealed in plastic bags and placed in a water bath at 44.5°F for 24 hours. The growth of *E. coli* using this processing method was similar to the number of colonies that grew using an incubator (Schraft, and Watterworth, 2006). This processing method may still require electricity in cold climates and uses water in a way that may be thought of as wasteful.

Level of training. This can be addressed depending on the method that is being implemented. Most of the presence/absence methods can be taught easily. However, methods that require aseptic technique, such as portable membrane filtration, are harder to teach. In these settings, maintaining sterile could be very hard to manage even if basic aseptic techniques are learned.

The MPN methods can be harder to interpret. Oftentimes, these tests are read in a lab by two or three scientists who have a lot of experience doing so. Multiple-reads are necessary to try to get the right count as, oftentimes, the colonies are counted differently. The color of the colonies can be hard to interpret as well and viewed differently by different people.

Furthermore, money for teaching methods may need to be included in a budget, and it should be a capital and recurring cost. More money will have be dedicated towards training from

a capital standpoint, but if the monitoring is to sustain for a long period of time, refresher trainings may become necessary. Even if money is included for teaching, the trainees will need experience along with training to become proficient at some of the methods. This should be considered and, if need be, an easier method chosen.

Cost. The cost of each test that can be used in low-resource settings needs to be considered from a capital and recurring standpoint as well. As mentioned above, for the example, many of the field tests function most accurately when they are incubated after processing. The price of incubators is extremely variable, but may be prohibitive regardless. One that is used by university researchers, for example, is made by Fisher scientific (catalog number 11-700-107) and costs $1,185.97, although these groups can benefit from a 10% discount. This incubator is decent size, digital, and only weighs approximately 20 lbs, which means it can be moved from testing site to testing site (Fisher Scientific, 2015).

There are cheaper incubators on the market. One recommended for use with Petrifilm, for example, is made by Nelson Jameson (model number 6426) and costs $471.26 USD. There is a smaller incubator, made by the same company (SKU 43100), which is only $93.49 and holds up to 40 petrifilm. An adapter can also be purchased to enable this incubator to plug into a dashboard outlet (Nelson Jameson, 2015). It is not necessarily realistic that settings will have a car with an outlet, or even a car at all, but it is another option to consider in places without electricity. Furthermore, if petrifilm is deemed the best test for a particular setting, the cheaper, smaller incubator may be sufficient for risk assessment. The incubators from Nelson Jameson carry a one to two year warranty, but should last around 10 years.

The Colilert® Quanti-Tray® has an additional cost of the sealer, which is approximately $4,000 USD (IDEXX, n.d). There is a low cost option to get around the need for a sealer,

however, although they both require electricity. An iron can be used to seal the tray. The tray should be covered with a cloth and the iron, set on the lowest temperature setting, should be run over the tray from the closed to the open side 5 times. Then, the iron should be held in place for 5 seconds working up from the closed end to open end. At the top, open end, it should be held there longer than 5 seconds to make sure the tray sealed. If it worked properly, the outline of each of the wells should be visible from the paper side (Lenczewski, 2013).

The individual cost of each testing method needs to be considered as well. The number of tests needed depends on the matrix of interest, the setting, and how many samples need to be run. They should not be compared generally, but, once a matrix is chosen, the capital and recurring costs for the potential methods that could be used can be considered. Although it may be more difficult to assess, perceived cost of each method should factored in as well to increase the chances of uptake and sustainability. Some of the methods are quicker than others, as well, and the desired turnaround time for the results should be considered with each setting in mind.

Flexibility. In conjunction with cost, flexibility of the testing method should be considered. Of the methods reviewed here, IDEXX, and Delagua are most easily adopted for use in testing for other organisms. *E. coli* is the bacteria of interest here but it may be necessary in low-resource settings to test for salmonella contamination in food or *Pseudomonas aeruginosa* in the water, for instance. IDEXX trays, for example, can be used to test for *Pseudomonas aeruginosa* by using the Pseudalert powder instead of Colilert® powder. If a company or group purchases an incubator and sealer, they would not be limited to only testing for *E. coli* with the IDEXX equipment. Similarly, Delagua can utilize different media to detect total coliforms (Delagua, n.d.). This flexibility reduces long term capital costs if one method can be adapted to recover a variety of organisms. Petrifilm, on the other hand, is less amenable to change. The

plates have to be purchased with media on them, but other plates that test for Salmonella or Yeast, for example, are available for purchase.

Number of Samples Needed. A final consideration when deciding which method to use is how many samples need to be processed in order to assess exposure. It is important to weigh the relative cost, in terms of the actual budget or supplies, versus desired precision. However, there will undoubtedly be variability in the levels of bacteria present, which means the number of samples needed may vary. One study done in the U.S. tried to develop a formula through statistical modeling for determining number of samples needed to achieve this balance. They mention difficulties, however, in accounting for temporal and spatial variability in the samples (Shukla, et. al, 2005). Water may be more contaminated depending on the season, for instance, or meat may be more contaminated depending on the skill of the person slaughtering the animal or cooking it. Therefore, each matrix and setting may have too many unique factors that cannot be controlled for and fit neatly into a formula. It can be difficult, therefore, to justify the details of the of the exposure assessment protocol to the community or stakeholders.

Budgetary constraints are usually one of the biggest factors in carrying out a study (Shukla, et. al, 2005). Once the budget is known, precision can be achieved by understanding which methods work best with the matrix of interest and gaining a preliminary understanding of expected contamination levels. This can be done through literature searches or initial testing on a small scale. It is important to be transparent about this variation and the sliding scale of samples that may be needed in order to maintain credibility with a community.

It is difficult to know ahead of time what the expected levels of contamination will be, how many samples will be run, and which bacteria should be targeted for detection. This may not affect capital cost, as an incubator is needed for most methods if there is money for purchasing

one, but it would likely add to the recurring cost. More forethought would be required as different plates would have to be ordered if a different test is needed.

One device, the WaterCanary, would solve a lot of these problems, at least for water testing, if it got onto the market for field use. A prototype of device is being evaluated for its use in low-resource settings. It does require electricity but does not require test strips, incubation, or reagents. It does not depend on chemical reactions, but utilizes light instead to indicate contamination. It would cut down on the number of samples needed and, therefore, the cost. It does not require advanced training; A sample of water is inserted in the device and, within seconds, a light on the top flashes red, for unsafe, or green, for safe (Luthra, 2011). It has networking hardware that provides real-time analysis of contamination levels and, when used without a water network, can identify where the problem areas are and create a map (WaterCanary, 2015). In smaller systems, water quality parameters can be set to continuously monitor the supply. At this point, it is only testing for turbidity as a proxy for contamination, but it still has potential for useful application in the field as a screen prior to microbial testing.

Chapter 4: Discussion, Recommendations, and Conclusion

4.1 Discussion

Limitations. There were a few limitations with this review. First of all, although the search was conducted systematically, it is possible that some relevant publications were missed. Search terms were thoughtfully chosen, but possibly not all inclusive. Furthermore, only 4 search engines were used and some articles may not have been accessible via these engines.

Secondly, the review may have been stronger if conducted as a meta-analysis. It would have been difficult, however, to combine the results of each testing method-specific study

together for any meaningful conclusion. Furthermore, the study goal was not amenable to a meta-analysis.

Thirdly, there is some bias in this review that was introduced by the inclusion and exclusion criteria. It is possible that relevant, novel, or conflicting results on this topic exist in literature not published in English, but unfortunately, non-English publications could not be reviewed due to the limitations of the reviewer. Furthermore, what constituted a low-resource setting and whether or not a study's setting fell into that category seems fairly straight forward, but could be considered subjective.

Gaps in Knowledge. This review has outlined plenty of options for water testing in low-resource settings. The tests that would work best for the other pathways of bacteria exposure is less clear, even after compiling all the literature. Also, there is a gap around conducting some of these tests truly without lab methods. Some of the described techniques for body incubation, and sealing with an iron seem theoretical at this point. More testing in the field should be done to document how well body incubation actually performs. Thirdly, there is a gap in knowledge and attitudes as to whether or not communities would use these techniques. Monitoring fecal contamination is important and documented in the literature, but it is unclear if communities in low-resource settings are aware and if they have a desire to ramp up monitoring. It would be important to assess this as it would be crucial to the uptake of test methods should they be adapted for low-resource settings.

4.2 Recommendations

Future Studies. More studies need to be done in the field to test hands, objects, food sand, soil, and fecal sludge. These studies need to be done in low-resource settings, in the field to

simulate the settings where testing capability is most needed. Whether they succeed or fail, results should be written up for development of the testing methods. Furthermore, as mentioned above, more of an effort should be made, through qualitative research, to understand how well monitoring is understood and whether or not it would be adopted.

It would also be useful to continue to make efforts towards statistically determining an appropriate number of samples to collect. The methodology of the study from 2005 by Shukla, et. al raised a lot of questions and likely cannot be applied to study designs now. But, oftentimes, the number of samples collected seems to be chosen randomly or *a priori.* If a more methodical way to arrive at a cost-effective, precise sample taking variability in account, it would be extremely beneficial.

Finally, a comparative study with each matrix in the future would be helpful. If all of these methods could be compared in one setting, allowing for the control of all other factors, it would provide the best picture of which method is best for each matrix. If this comparative study could be done, the reliability and robustness of each method could be documented as well. In low-resource settings, these characteristics would be especially important as circumstances are always subject to change. A few articles started to discuss robustness of the Petrifilm and IDEXX in terms of temperature and time, but there is not yet enough data to know if the performance of these methods is as expected in less than perfect temperatures and time. It would also be beneficial to know, for example, how the ability to maintain sterility affects the methods.

Matrix-Specific recommendations.

1. **Water**: IDEXX Quanti-tray/Quanti-tray-2000 seems to offer the most ease in testing large quantities of water. If expected levels of contamination are unknown, it is the ideal

method because multiple dilutions are not needed and level of training is low. Furthermore, if no electricity is available and the processing equipment too expensive, it can still be used with body incubation and an iron. If the IDEXX equipment is deemed too expensive or the generated waste cannot be dealt with, the second choice recommended based on this literature would be CBT. Furthermore, if testing is being done on an improved water source, Petrifilm can be used without electricity.

2. **Produce**: Most the literature supports the use of Petrifilm for produce testing but this method requires more skill to prepare and interpret. It could require multiple dilutions, also, which can get expensive. IDEXX or CBT could be used in settings without electricity and/or an incubator. Although CBT has not theoretically been used for produce testing, the produce should be rinsed in a sample bag, and that rinse becomes the sample, which could be tested in CBT or IDEXX.
3. **Fecal Sludge:** IDEXX was documented in the literature as a useful method to use to recover *E. coli* from sludge. But the literature was not extensive and the studies were not all truly field-based. The same financial, training, equipment, and electricity constraints need to be considered. IDEXX would likely be the easiest to use if contamination levels are unknown, and personnel are less skilled. If the equipment for IDEXX, the sealer and an incubator, can be purchased, then other pathogens besides *E. coli* could likely be recovered. The literature supporting the recovery of other organisms in fecal sludge, however, was not researched here. If high levels of contamination are expected, then Petrifilm could also be used. They requires less incubator space, and the capital costs are lower, but more studies are needed to test the efficacy of *E. coli* recovery in the matrix

using Petrifilm. CBT might be useful for testing, but this method would also require more studies with feces or fecal sludge.

4. **Hands:** Petrifilm is the best method for testing hand contamination per the literature. The same problem applies: Petrifilm is most effective in this matrix if high levels of contamination are expected. If only a small level of contamination is expected, however, or the test comes back negative, it would be necessary to take hand rinses and test them with IDEXX or CBT. Any of these three methods can be processed without an incubator if it cannot be purchased or there is no electricity. Hands can be tested with IDEXX or CBT by rinsing/dipping the hand into a bag with distilled water, massaging them, and using that water as the sample. This would likely be more feasible in low-resource field settings than Petrifilm.
5. **Objects:** For objects, such as toys, utensils, and medical devices, the same methods should be utilized as with hands or produce. There is not an extensive amount of literature where *E. coli* is recovered from objects but the studies that do exist support the use of rinses using IDEXX. Therefore, despite other considerations, IDEXX or CBT should be used unless further research is done in the future using other methods.
6. **Meat:** Petrifilm is the best method to use when testing meat products according to the studies reviewed here. The processing steps can be complex, but it is the preferred method mentioned for recovery of *E. coli* from meat if it can be adapted for field use. Further research would be needed to see if rinses, used with IDEXX or CBT, could be used to recover *E. coli* from meat. If so, these methods would be useful as they require less training.

7. **Sand/Soil:** There was not an extensive amount of literature demonstrating *E. coli* recovery in sand or soil. Soil can be tested using Petrifilm, but the literature showing this was not done in the field. Sand requires further testing to determine which method would be the best. More research for both matrices should be done in low-resource for appropriate ways to test for contamination. Currently, the small amount of studies found point to portable membrane filtration techniques, such as Delagua, being most useful for testing sand.

4.3 Conclusion

Testing for contamination in low-resource settings can be done. The challenges that exist are real, but not insurmountable. As this review demonstrates, the methods that exist for testing can be adapted for use outside of a traditional lab setting. The level of trained personnel, presence of electricity, budget, level of contamination, matrix, and equipment needs all have to be considered but these parameters will aid in choosing the right method. Specificity may be reduced in the field but it remains high enough to identify high risk exposures, as studies suggest that only highly contaminated water (100 cfu/100 mls or greater) is associated with diarrhea. Exposure to pathogens and the illness that follows hinders development in low-resource settings and reduces contributions from the sick. The morbidity and mortality from WASH-associated diseases are preventable and failing to monitor risk in low-resource settings due to the absence of a laboratory is unacceptable. Furthermore, water monitoring is likely to be included in the post-2015 development agenda that is building off of the Millennium Development Goals, pressuring governments to monitor regardless of resources.

Public health practitioners should make every effort to incorporate field testing into their projects. Demonstrating field techniques and the results that are obtained is extremely important for implementing a monitoring plan in low-resource settings. Furthermore, teaching these techniques to people in low-resource settings for regular use will increase sustainability. If sustainable monitoring plans could be developed with the help of field testing techniques, progress will be made in improving the health of communities.

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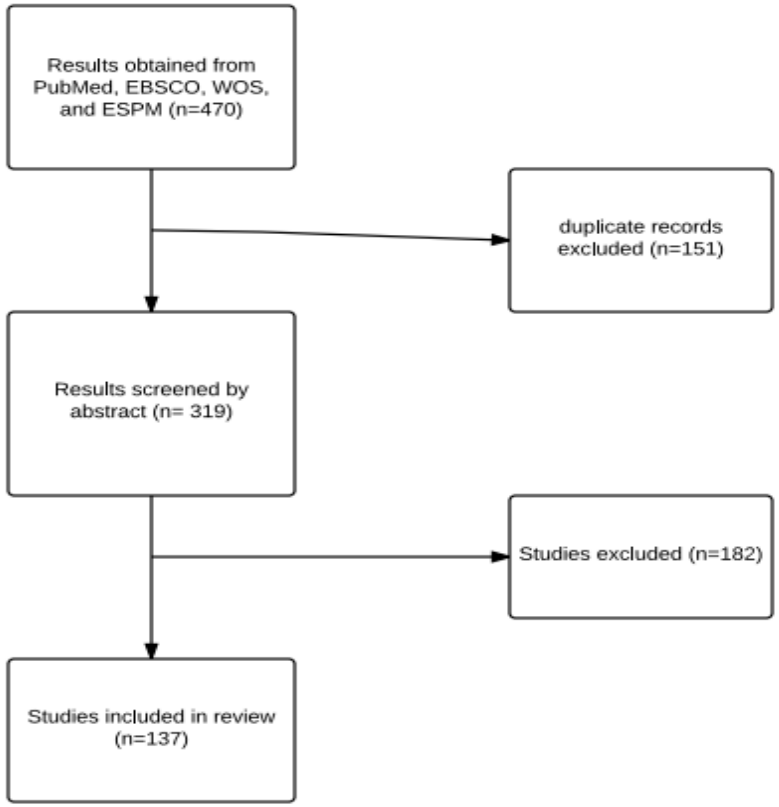
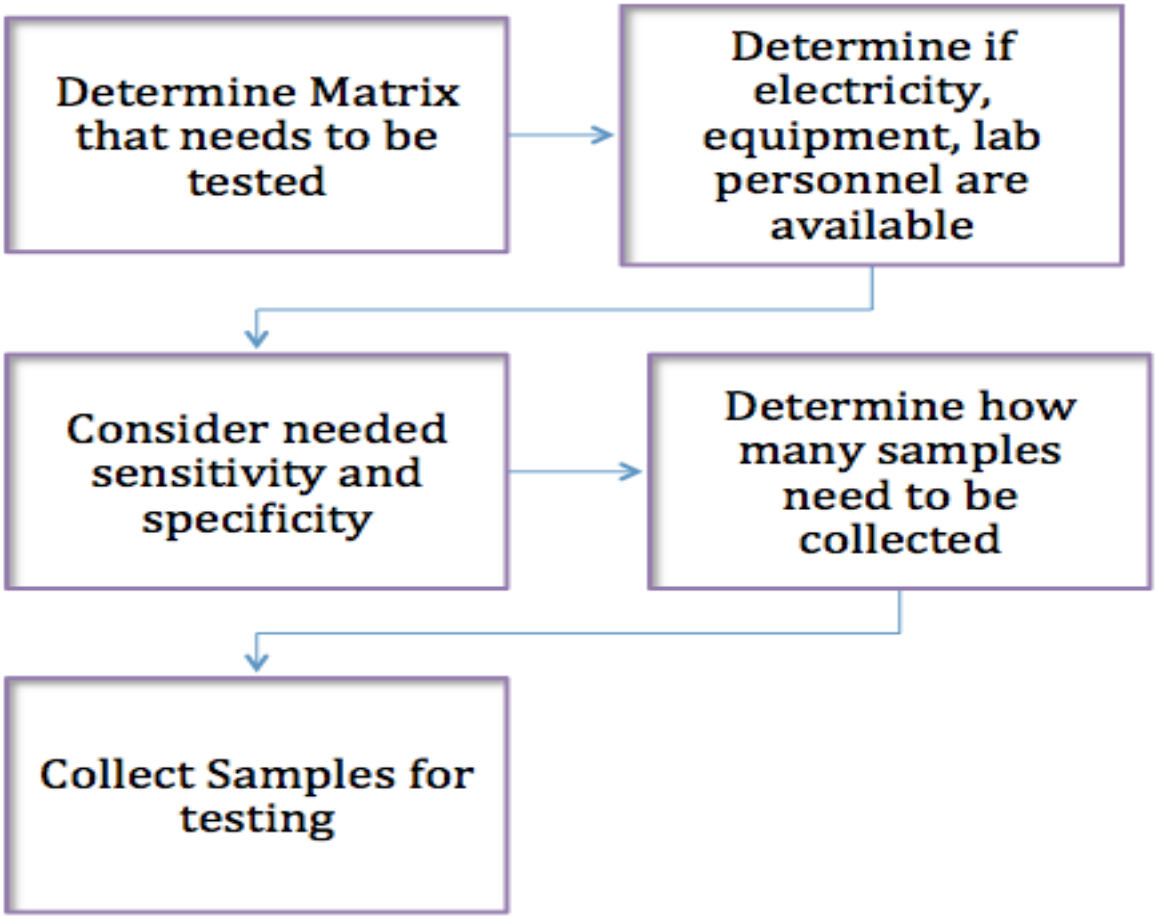
Appendix 1. Article flowchart

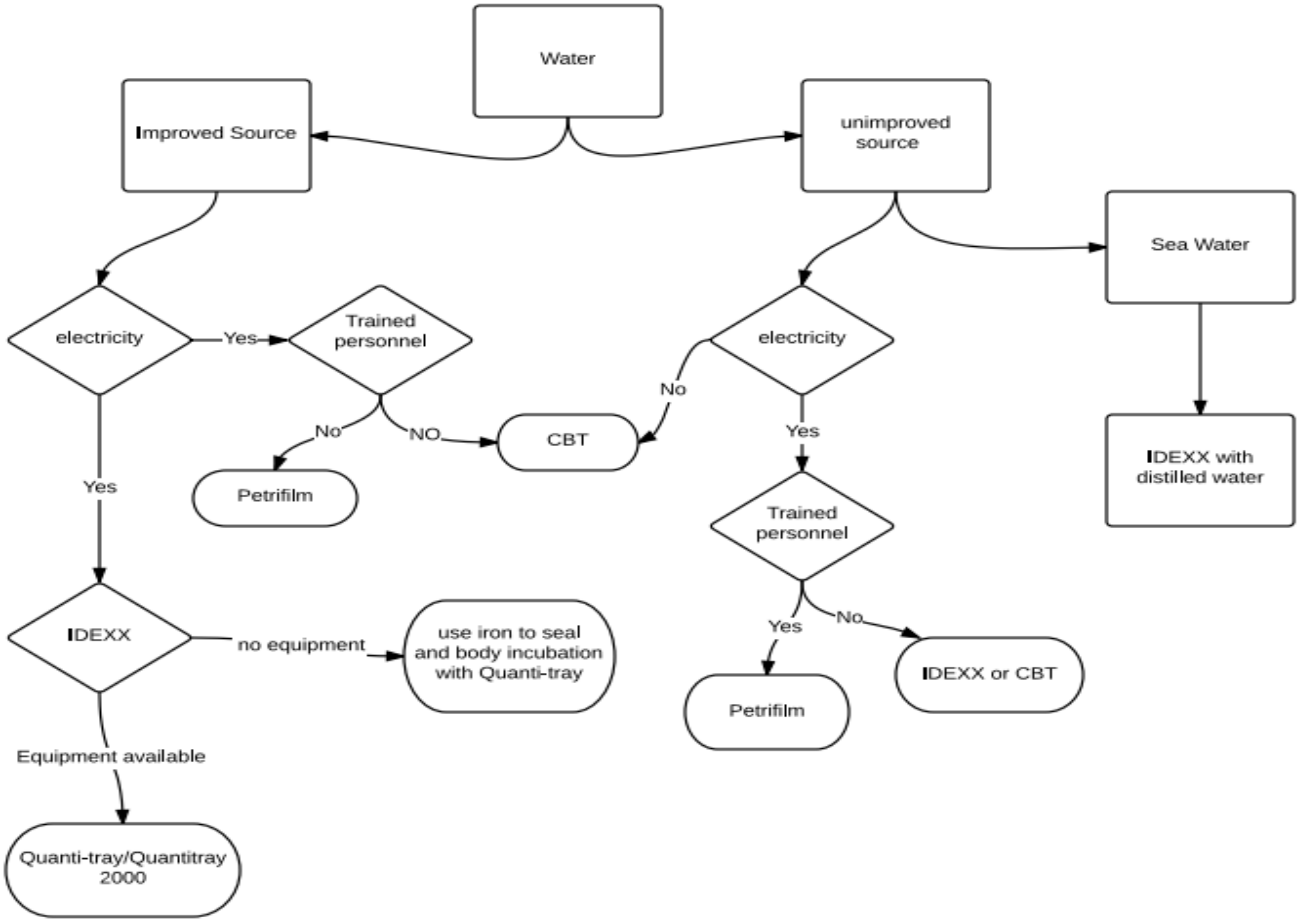
Figure 1. Article selection flowchart



Appendix 2. General decision flowchart for testing



Appendix 3. Flow diagram for choosing water method



On the unimproved side, the Colilert® method should used if electricity is present, but trained personnel are not regardless of Colilert® equipment availability.

Appendix 4. Flow chart for choosing method with produce