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EVALUATION OF THE COMPARTMENT BAG TEST FOR THE DETECTION OF *ESCHERICHIA COLI* IN DRINKING WATER

by

CANDACE MILLER

M.S., UNIVERSITY OF ALABAMA AT BIRMINGHAM
B.S., SPELMAN COLLEGE

A Thesis Submitted to the Graduate Faculty
of Georgia State University in Partial Fulfillment
of the
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ATLANTA, GEORGIA 30303

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APPROVAL PAGE

EVALUATION OF THE COMPARTMENT BAG TEST FOR THE DETECTION OF *ESCHERICHIA COLI* IN DRINKING WATER

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In conclusion, I would like to acknowledge that this research would not have been possible without the scientific contributions of researchers at the University of North Carolina at Chapel Hill, and the support provided by the Research Initiation Grant and the Georgia State University Institute of Public Health.

ABSTRACT

INTRODUCTION. More than 1.8 million diarrheal disease deaths can be attributed to the lack of access to water, sanitation and hygiene. These deaths occur mostly in developing countries where water quality testing resources are limited. Several tests are currently used to detect and quantify *E. coli* and other fecal coliforms in drinking water, however they can be expensive, complex, and technically demanding. There is a need for a simple, reliable, low-cost water quality test that can be used in resource limited settings. Therefore, the purpose of this research was to perform a rigorous evaluation of the recently developed compartment bag test for detection and quantification of *E. coli* against the standard method, membrane filtration.

METHODS AND RESULTS. A total of 270 water samples were collected from forty-five various naturally contaminated water sources around metro-Atlanta from August 2011 through April 2012 and processed using the compartment bag test and membrane filtration with mI agar. Concentrations of *E. coli* were significantly correlated with a correlation coefficient of 0.904 (95% CI 0.859 – 0.950). Sensitivity and specificity were 94.9% and 96.6%, respectively.

CONCLUSION. These results suggest that the compartment bag test produces results consistent with those produced by membrane filtration on mI agar. Based upon its performance, the compartment bag test has the potential to be used as a reliable, low-cost drinking water quality test globally where water quality testing resources are not readily available, and can be implemented in monitoring activities for microbial water quality.

KEYWORDS
Environmental health, drinking water quality, microbial water testing, fecal indicator, *E. coli*, safe water

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CHAPTER I

INTRODUCTION

Approximately 783 million people worldwide do not have access to safe drinking water, with an estimated 2.5 billion people lacking access to sanitation, placing them at risk for waterborne illnesses. Diarrheal diseases cause more morbidity and mortality in children than malaria, AIDS, and measles collectively, resulting in the second leading cause of death globally among children under the age of five (Liu et al., 2011). One of the major causes of diarrheal disease is the consumption of microbiologically contaminated drinking water. Access to tests needed to monitor water quality and microbiological safety is limited in developing countries. The ability to provide a reliable, simple, low-cost test in limited resource settings may reduce the consumption of unsafe drinking water, thereby reducing the risk of waterborne disease.

Current drinking water quality tests are expensive, complex, and require extraneous laboratory equipment. Due to the immediate need for a simple, low-cost water quality test the compartment bag test has been developed in collaboration with researchers at the University of North Carolina. It is portable, and does not require extensive and expensive laboratory equipment that is normally needed for current tests of

drinking water quality. This test can be performed in developing countries with limited resources, as well as in developed countries in the occurrence of natural disasters. As a result of being recently developed, the compartment bag test needs significant validation in order to increase scientific evidence and be widely accepted by the scientific community.

This research was conducted to subject the compartment bag test to rigorous performance testing against the standard method, membrane filtration with mI agar. Information obtained from this study will help to promote the application of this test in resource limited settings. The compartment bag test has the potential to aid in the prevention of diarrheal disease, support water safety plans, and serve as a tool in microbial water quality monitoring activities.

CHAPTER II

LITERATURE REVIEW

Overview

Diarrheal diseases are the second leading cause of death among children under the age of five (Liu et al., 2012). It is estimated that 88% of deaths resulting from diarrheal diseases can be attributed to poor sanitation and hygiene, and the consumption of unsafe drinking water. Approximately one-half of developing countries lack access to safe drinking water (WHO/GLAAS, 2012). Due to the devastating impact of diarrheal disease in the developing world, there is a need for improved drinking water quality tests that can not only help prevent diarrheal disease, but are also economically feasible when resources are limited. The current tests for the detection of *Escherichia coli* (*E. coli*) in drinking water are complex and difficult to perform (Boubetra, Le Nestour, Allaert, & Feinberg, 2011). They require laboratory equipment and materials that may not be readily available in resource limited settings, and can be less affordable for developing countries (Sobsey & Pfaender, 2002).

The compartment bag test is a low-cost test that is simple and easy to use. It is portable, and does not require extensive and expensive laboratory equipment that is normally needed for current tests of drinking water quality. The compartment bag test consists of a sterile, clear, disposal plastic bag into which the water sample of interest and chromogenic media are poured into. This test is scientific based, and has the potential of being used worldwide in resource limited settings. However, because it is a newly

developed test, there is still a need for rigorous validation to provide more evidence of its effectiveness, efficacy, and efficiency. The potential impact of the compartment bag test is vast due to the substantial global burden of infectious diarrhea and other enteric diseases.

Epidemiology of Diarrheal Disease

Burden of Diarrheal Disease

Diarrheal diseases are responsible for causing high rates of morbidity and mortality in developing countries (Bryce, Boschi-Pinto, Shibuya, & Black, 2005). They are more likely to occur in situations where there is a lack of adequate water supplies, and poor sanitation and hygiene. Globally, roughly 783 million people lack access to improved drinking water, and over two billion have no basic sanitation (WHO/GLAAS, 2012).

The World Health Organization reports approximately 2.2 million diarrheal deaths each year among children under the age of five in developing countries. Consumption of unsafe drinking water takes a toll on both human health and individual productivity through the development of diarrheal diseases (Prüss-Üstün & Corvalán, 2006). It is estimated that diarrheal diseases amount to 4.1% of the total disability-adjusted life year (DALY) global burden of disease. More than four-fifths of this burden is due to unsafe drinking water, sanitation, and hygiene (WHO/GLAAS, 2012).

The overall diarrheal disease case fatality rate in children less than 5 years of age is 0.2% in developing countries, being the highest in the youngest children. The case fatality rates in children in developing countries range from 0.1% to 0.5% in settings that are as diverse as the rural areas of Indonesia, rural Egypt, urban Central African Republic, and rural North India (Bhan MK & K, 1989).

In developing countries, the median annual incidence of diarrheal disease in children under 5 years of age was 3.5 episodes (WHO/UNICEF, 2009). The incidence was highest in community-based studies with a small number of children under surveillance, and with more frequent home visiting. This suggests that other studies may have found lower rates due to underreporting. The incidence of diarrheal disease varies based upon age. The highest incidence is seen in children 2 years old or younger, with a peak seen at 6 to 17 months of age. However, this declines with aging (Bern, Martines, De Zoysa, & Glass, 1992).

Diarrheal diseases have unfavorable effects on the growth of children in developing countries. This is due to decreased absorption of nutrients, decreased appetite, and in some cases a changed diet. Diarrheal disease in children within the first several months of life can result in more long-term growth deficiencies than in older children (Black, 1991).

In developing countries, diarrheal diseases are also an economic burden due to the costs of medical care and the loss of daily life functionality. There have been increased

efforts to reduce diarrheal mortality as the severity of diarrheal disease is increasing in some countries. This has led to more research being conducted on ways to prevent diarrheal disease through the development of low-cost drinking water quality tests.

Drinking Water Quality and Diarrheal Disease

One of the major causes of diarrheal disease is the consumption of contaminated drinking water. Fecal coliforms, particularly *E. coli*, have been used as indicators of fecal contamination of drinking water (Horan, 2003). The presence of *E. coli* is customarily associated with fecal contamination because it is the most common fecal coliform among the intestinal flora of warm-blooded animals (Rompré, Servais, Baudart, de-Roubin, & Laurent, 2002). Contaminated drinking water may contain unsafe levels of microorganisms that pose a risk to human health (JMP, 2010). In many developing countries the use of untreated water sources makes the assessment of fecal contamination of drinking water chiefly important (Fewtrell & Bartram, 2001). Moe et al. (1991) found that when the water supply contained greater than 1,000 *E. coli* per 100 mL of water the rate of diarrheal disease was significantly higher. As a result contaminated water becomes a major source of exposure to fecal contamination and diarrheal pathogens. The study concluded that compared to other bacterial indicators, *E. coli* appears to be a better predictor of the risk of waterborne gastrointestinal illness (Moe, Sobsey, Samsa, & Mesolo, 1991). The World Health Organization has specified that zero *E. coli* per 100 mL of water is the goal for all water supplies and should be the target even in emergency situations (WHO, 2006).

There are currently several tests used to detect and quantify *E. coli* and other fecal coliforms in drinking water, however they can be expensive, complex, and time consuming. In addition, the lack of access to water analysis kits or laboratories is an issue for many communities in the developing world (Sundram, Bailey, & Green, 2000). There is a need for simple, reliable, low-cost interventions that will be readily available to people in developing countries with limited access to water, sanitation, and hygiene, therefore helping to prevent diarrheal disease (McMahan, Devine, Grunden, & Sobsey, 2011).

Comparison of Water Quality Tests

Membrane Filtration

Membrane filtration is a traditional technique used for the microbial analysis of coliform bacteria in water samples. It is used for both the detection and enumeration of fecal coliforms in drinking water (Rompré et al., 2002). In many countries this technique is approved and accepted as a common procedure for monitoring the microbial quality of drinking water.

The technique consists of filtering a predetermined volume, principally 100 mL, of a water sample through a filter funnel onto a sterile membrane with a 0.47µm pore size via vacuum pressure. Any microorganisms present in the sample are concentrated on the membrane surface. After complete filtration of the water sample, the filter is placed on

selective media and incubated at 35-37°C for 24 hours. After incubation, typical colony growth on the filter is enumerated. The colonies that form on the membrane surface can be transferred to other microbiological media to further confirm identification.

One of the selective media commonly used with the membrane filtration technique is MI agar medium. This medium was developed by Brenner et al. (1993) to concurrently detect total coliforms and *E. coli* in water samples. The medium contains flurogenic 4-methylumbelliferyl-beta-D-galactopyranoside (MUGal) and chromogenic indoxyl-beta-D-glucuronide (IBDG). The total coliforms produce beta-galactosidase cleaving MUGal which fluoresces under UV light, whereas *E. coli* produces beta-glucuronidase which cleaves IBDG forming a bright blue color (Brenner et al., 1993).

The technique was shown to be sensitive, selective, specific, and rapid with low false positive and false negative rates by Brenner et al. (1993). Specificity for *E. coli* on MI agar was 95.7% which is similar to the specificity reported for other media used with membrane filtration in Hartman (1989), but differs from the results found in Chang et al. (1989). The false negative and false positive rates were both 4.3% (Brenner et al., 1993).

The membrane filtration technique, with the use of MI agar, may produce results within a turnaround time of 24 hours. The method requires approximately 15 minutes of hands-on time, but must be incubated for at least 18-24 hours. The materials needed for membrane filtration can be considered to be expensive at $2,500 for the membrane filtration assembly including the vacuum pump, costing $1.70 per test (Bain et al., 2012).

Competent laboratory personnel are required to perform membrane filtration using MI agar without interpretation problems.

Quality control procedures must be used for membrane filtration with MI agar. Each new batch of MI agar plates must be tested using a known *E. coli* isolate as the positive control, and phosphate-buffer saline for the negative control. The MI agar plates must be used within the specified expiration time to be able to fully observe colony color production. If bacterial growth is observed for the negative control, this may be due to contamination of the materials.

An advantage of membrane filtration is that it is feasible to examine larger volumes of water when necessary. It also provides quantitative rather than semi-quantitative enumeration. Membrane filtration is a useful technique for many water quality laboratories in that many samples can be processed with limited staff. It is less expensive than liquid chromogen and fluorogen media. However, a wide range of basic laboratory equipment is required which can be expensive and not readily available in resource limited settings. In addition to this, the technique requires skilled laboratory personnel with microbiological training. The plates also may not be read and interpreted the same way by various laboratory personnel observing the plates. Some colonies may be misidentified due to variation in color.

Compartment Bag Test

The compartment bag test is a simple, low-cost test for drinking water quality that has been recently developed in collaboration with researchers at the University of North Carolina at Chapel Hill. It is able to detect and quantify *E. coli* based upon the most probable number principle. This test is portable, affordable, and can be used without extensive laboratory equipment that is normally needed for standard tests for drinking water quality.

The components of the compartment bag test include a clear, sterile, disposable plastic bag, dry chromogenic substrate culture media, and a water collection container. The test consists of mixing the chromogenic medium with up to 100 mL of the water sample, pouring it into the compartment bag, and gently squeezing the bag to distribute the correct volumes into the designated compartments. The bag is then sealed with a reusable plastic clip and incubated for approximately 24 hours. With the use of chromogenic substrate culture media, *E. coli* can be detected through the production of a blue-green color change in the water sample due to the utilization of the specific substrate in the medium.

A study by McMahan et al. found that the performance characteristics of the compartment bag test were notable in comparison to the Quanit-Tray IDEXX Colilert® assay. Sensitivity, Specificity, NPV, and PPV were 73%, 100%, 68%, and 100%, respectively for the compartment bag test when observed after 24 hours of incubation

(McMahan et al.). The test produced a false positive rate of 0% and a false negative rate of 27%.

The compartment bag test has a turnaround time of 24-48 hours. It requires less than 5 minutes of hands-on time, and can be performed with minimal materials as there is no need for special laboratory equipment. It is suitable for low resource settings. Cost per test is $1.00 (Bain et al., 2012). The test is non-complex and does not require the use of trained laboratory personnel. A negative control can be prepared using phosphate-buffered saline to ensure the absence of contamination.

Advantages to using the compartment bag test are less hands-on time, affordability, and fewer resources required. The test can be easily performed by someone without laboratory training. Expensive materials and equipment are not required for the test, and therefore can be used in remote settings where resources are limited. Unlike many other drinking water quality tests the compartment bag test does not require the use of electricity, utilizing ambient temperature incubation or the use of a warming agent that does not require electricity. This compact, simple test is able to help prevent exposure to enteric pathogens, and has the potential to provide water quality managers, disaster relief officials, and water, sanitation and hygiene staff with rapid, reliable results for decisions necessary to protect the public from waterborne infectious diseases.

Table 1. Comparison of Water Quality Tests

	Membrane Filtration	IDEXX Colilert® Quanti-Tray	Compartment Bag Test
Hands-on Time	15 minutes	10 minutes	5 minutes
Turnaround Time	24 hours	24 hours	24 hours
Cost	$1.70 per test $2,500 (equipment)	$5.50 per test $6,000 (equipment)	$1.00 per test $0 (equipment)
Level of Complexity	High	Moderate	Low
Testing Environment	Laboratory	Laboratory	Non-Laboratory
Resource Setting	High resource	High resource	Low resource

Purpose of Research

There are several drinking water quality tests available that are applicable in various field settings, however they do not meet the needs in resource limited settings due to cost and the necessity of large volumes of water to be sampled (Brown et al., 2011). The compartment bag test has been developed as a less expensive and simple to use test to detect the presence of *E. coli* in drinking water. Due to being recently developed, this research was necessary to evaluate the compartment bag test for the detection and quantification of *E. coli* in drinking water against the standard method, membrane filtration using validation and verification protocols. Increasing the evidence for the compartment bag test will provide an opportunity to be widely accepted by the scientific

community. This will help promote the application of this test not only in developing countries, but also in developed countries when access to safe drinking water is limited in the event of natural disasters.

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CHAPTER III

MANUSCRIPT

INTRODUCTION

Globally, roughly 783 million people lack access to improved drinking water, and over two billion have no basic sanitation (WHO/GLAAS, 2012). There are approximately two billion cases of diarrheal disease globally every year. It is a leading cause of preventable deaths worldwide and the second leading cause of mortality and morbidity in children under the age of five (Liu et al., 2012). One of the major causes of the 2.2 million deaths due to diarrheal disease is the consumption of contaminated water (WHO/UNICEF, 2009).

The microbial quality of water has a large impact on health in developing countries where access to safe drinking water is limited (Fewtrell & Bartram, 2001). Contaminated drinking water may contain unsafe levels of microorganisms that pose a risk to human health (JMP, 2010). Fecal coliforms, particularly *Escherichia coli* (*E. coli*), have been used as indicators of fecal contamination of drinking water (Horan, 2003). The World Health Organization has specified that zero *E. coli* per 100 mL of water is the goal for all water supplies (WHO, 2006). There are several current tests used to detect and quantify *E. coli* and other fecal coliforms in drinking water, however they

can be expensive, complex, and time consuming (Boubetra, Le Nestour, Allaert, & Feinberg, 2011). Most of these tests require trained laboratory personnel and a laboratory setting that may not be available in remote areas (NRC, 2004). In addition, the lack of access to water analysis kits or laboratories is an issue for many communities in the developing world (Sundram, Bailey, & Green, 2000).

There is a need for simple, reliable, low-cost interventions that will be readily available to people in developing countries with limited access to water, sanitation, and hygiene, therefore helping to prevent diarrheal disease (McMahan, Devine, Grunden, & Sobsey, 2011). As a result, the compartment bag test has been developed as a less expensive and simple to use test to detect the presence of *E. coli* in drinking water. The purpose of this study was to evaluate the compartment bag test for the detection and quantification of *E. coli* in drinking water against the standard method, membrane filtration using mI agar for various natural water sources in Atlanta, GA.

MATERIALS AND METHODS

Water samples were collected from forty-five various naturally contaminated water sources around metro-Atlanta. The samples were collected from August 2011 through April 2012 as a part of the Research Initiation Grant in collaboration with the University of North Carolina at Chapel Hill. Each sample was labeled with the location

of the water source and date of collection, stored at 2-8°C, and processed within 24 hours of collection. A total of 270 samples were processed and tested in the Institute of Public Health Laboratory at Georgia State University using the compartment bag test and the traditional membrane filtration method.

Membrane Filtration Method

Water sample volumes of 10, 50, or 100 mL were processed in duplicates using membrane filtration and selective medium (mI agar, Becton Dickinson, Sparks, MD) containing chromogenic and fluorogenic β-glucuronide and β-galactoside substrates for the detection and enumeration of *E. coli* following standard method 1604 (EPA, 2002). After adherence of the membrane to the agar, the plates were inverted and incubated for 18-24 hours at 35°C. *Escherichia coli* colonies were quantified and reported as colony-forming units (CFU) per 100 mL (EPA, 2002).

Compartment Bag Test Method

A total volume of 10, 50, or 100 mL of each water sample was mixed with a chromogenic *E. coli* broth culture medium and poured into a clear, standard water sample collection bag consisting of 5 internal compartments with individual volumes of 1, 3, 10, 30, and 56 mL each. After filling the compartmentalized plastic bags, the sample was manually distributed into each compartment by gently squeezing to ensure that each sample volume was filled to the set mark (Figure 1). Each bag was then sealed using a

two-piece plastic clip, with isolation of each compartment, and incubated for 18-24 hours at 35°C. After incubation, each compartment of the bag was scored. Concentrations of *E. coli* were determined using positive and negative compartment bag combinations which correspond to specified most probable number (MPN) values. Positive compartments of the bag were identified as those which turned a blue-green color, indicating the presence of *E. coli* due to the hydrolysis of the β-glucuronide substrate (McMahan et al.). This is based upon the principle that most *E. coli* strains produce β-glucuronidase (Watkins, Rippey, Clavet, Kelley-Reitz, & Burkhardt, 1988). Indeterminate results were noted and re-evaluated.

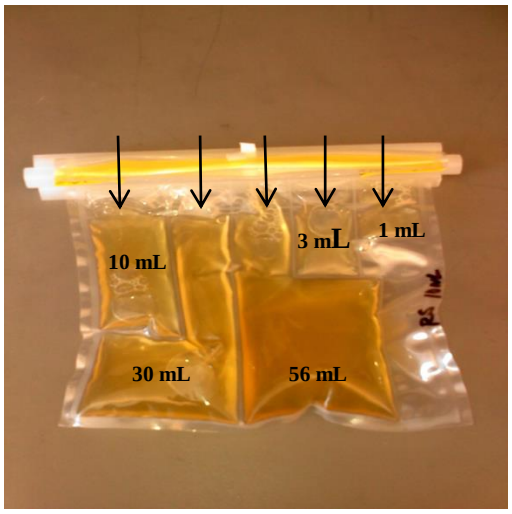

Figure 1. Compartment Bag Test

Isolation and Purification of Presumptive *E. coli* Positives

Presumptive *E. coli* isolates from positive compartments of the compartment bag test and isolates from the mI plates were streaked for isolation to Trypticase Soy Agar (TSA) and Bio-Rad RAPID'*E.coli* 2™ agar to obtain pure isolates and for organism identification for 123 of the water samples. For each compartment bag test with at least

one positive compartment, the smallest volume compartment that turned positive was first streaked for isolation onto Bio-Rad RAPID'*E.coli* 2™ agar and incubated for 18-24 hours at 45°C. A colony with the typical appearance of *E. coli* from each of these plates was then re-streaked onto another plate of Bio-Rad RAPID'*E.coli* 2™ agar and incubated for 18-24 hours at 45°C to ensure a pure culture. Colonies with the typical appearance of *E. coli* were then streaked onto TSA and incubated for 18-24 hours at 35°C. Pure isolated colonies from the TSA plates were added to individual 1mL aliquots of 20% glycerol and TSB and stored at -80°C for future use. Frozen isolates were thawed and streaked onto TSA for further identification. Organism identification was confirmed with the pure isolates using the BBL™ Enterotube™ II multiple biochemical test system for the identification of Enterobacteriaceae.

Data Analysis

The data for each water sample were recorded and entered into Microsoft Excel and copied into Stata 10.0 (StataCorp, College Station, Texas, USA) for further analysis. The water quality testing results from membrane filtration and the compartment bag test were characterized using descriptive statistics. These included geometric and arithmetic means with 95% confidence intervals, variance, and standard deviation using categorical and continuous data in both $\log_{10}$ transformed and non-$\log_{10}$ transformed format. Correlation analysis was used to determine how the two methods compared with each other for the same water sample based on the presence and concentration of *E.coli*. This included correlations between estimates for *E. coli* within the categories commonly used

to indicate fecal contamination and the level of waterborne disease risk as specified by the World Health Organization (WHO, 2006). Spearman's rank correlation coefficient was used to measure how closely the *E. coli* concentrations for the two methods compare. The compartment bag test and membrane filtration method were also compared on the basis of sensitivity [(true positives) / (true positives + false negatives)] and specificity [(true negatives) / (true negatives + false positives)] for the presence of *E. coli* compared to a gold standard of biochemical identification.

Parametric and non-parametric statistical tests were used to compare results where data were and were not normally distributed. Using continuous and $\log_{10}$ transformed data comparisons were made with mean comparison tests. A Wilcoxon Rank Sum test was also performed to compare the two methods and determine if the medians were statistically different.

RESULTS

A total of 270 naturally contaminated urban water samples were tested and grouped by type of water source (Table 2). The geometric and arithmetic mean *E. coli* counts were 1.52 (95% CI 1.39 – 1.64) MPN/100 mL and 212.4 (95% CI 170.5 – 254.2) MPN/100 mL, respectively for the compartment bag test, versus 1.63 (95% CI 1.51 – 1.76) CFU/100 mL and 232.5 (95% CI 190.7 – 274.3) CFU/100 mL for membrane

filtration. The Wilcoxon Rank Sum test for paired samples revealed that the median concentrations of *E. coli* were significantly different between methods (p = 0.0002).

Table 2. Descriptive Characteristics of Urban Water Samples Collected Between August 2011 and April 2012

Type of Source	Number of Samples	Season	Total
Creek	18	Summer	84
	22	Fall	
	24	Winter	
	20	Spring	
Lake	22	Summer	86
	27	Fall	
	17	Winter	
	20	Spring	
Pond	4	Winter	9
	5	Spring	
Stream	1	Summer	24
	3	Fall	
	8	Winter	
	12	Spring	
Rainwater	6	Summer	64
	42	Fall	
	8	Winter	
	8	Spring	
Other	3	Winter	3

As shown in Table 3, *E. coli* concentrations for the two methods were significantly correlated with a correlation coefficient of 0.904 (95% CI 0.859 – 0.950) when linear regression was performed. Furthermore, when compared on categorical *E. coli* concentration basis, there was fairly high agreement, especially at higher

concentrations as shown in Table 4. As specified by the World Health Organization, these categories [<1 CFU/100 mL (very low risk), 1-9 CFU/100 mL (low risk), 10-99 CFU/100 mL (moderate risk), ≥100 CFU/ 100 mL (high risk)] are used to indicate fecal contamination and the level of waterborne disease risk.

Table 3. Results from Linear Regression Measuring *E. coli* Concentrations of the Compartment Bag Test and Membrane Filtration

Membrane Filtration (CFU/100 mL)	Coefficient	Standard Error	t	P	95% CI	Spearman's rho
CBT (MPN/100 mL)	0.904	0.0233	38.89	<0.001	0.8588 – 0.9504	0.9278

Table 4. Comparison of Categorical Concentrations of *E. coli* for the Compartment Bag Test (CBT) and Membrane Filtration

	Membrane Filtration (CFU/100 mL)				
CBT (MPN/100 mL)	**<1**	**1-9**	**10-99**	**≥100**	**Total**
<1	28 70.0%	8 20.0%	4 10.0%	0 0%	40 100%
1-9	1 2.38%	30 71.4%	9 21.4%	2 4.76%	42 100%
10-99	0 0%	7 10.3%	50 73.5%	11 16.2%	68 100%
≥100	0 0%	0 0%	8 7.21%	103 92.8%	111 100%
Total	29 11.1%	45 17.2%	71 27.2%	116 44.4%	261 100%

Out of 263 samples tested with both methods, 12 (30%) were negative for *E. coli* (<1 MPN/100 mL) when tested with the compartment bag test, whereas the same samples were positive for *E. coli* (1 - > 100 CFU/100 mL) when tested with membrane filtration. These were considered to be false negatives. One (0.45%) sample was considered to be a false positive as it was positive for *E. coli* when tested using the compartment bag test, but negative for *E. coli* when tested using membrane filtration. We found that the compartment bag test produced a sensitivity of 94.9% and a specificity of 96.6% when compared with membrane filtration on mI agar (Tables 5 and 6).

Table 5. Statistical Comparison of Results for the Compartment Bag Test Compared to the Standard Method, Membrane Filtration

	Membrane Filtration		
CBT	**Negative**	**Positive**	**Total**
Negative	28 70%	12 30%	40 100%
Positive	1 0.45%	222 99.6%	223 100%
Total	29 11%	234 89%	263 100%

Table 6. Performance Characteristics of the Compartment Bag Test for the Detection of *E. coli*

Sensitivity	94.9%
Specificity	96.6%
PPV	99.6%
NPV	70.0%
False Positive Rate	3.4%
False Negative Rate	5.1%
Accuracy	95%

The Enterotube™ II biochemical test showed that 100% (43 out of 43) of the presumptive *E. coli* isolates obtained from the compartment bag test were identified as *E. coli* (Table 7). Out of 109 presumptive *E. coli* isolates from the compartment bag test (43 presumptive *E. coli* isolates) and membrane filtration (66 presumptive *E. coli* isolates), a total of 108 isolates were identified as *E. coli*. Isolates from both the compartment bag test and membrane filtration not presumed to be *E. coli* were also biochemically tested. Based on the biochemical analysis of presumptive *E. coli* isolates, 99% were identified as *E. coli* with one isolate (1%) identified as *Enterobacter aerogenes.*

Table 7. Enterotube™ II Biochemical Test Results for Presumptive *E. coli* Isolates

Type of Source	Number of Isolates Identified as *E. coli*		Total Number of Isolates Identified as *E. coli*
	Compartment Bag Test	**Membrane Filtration**	
Creek	21 100%	27 96%	48 98%
Lake	18 100%	31 100%	49 100%
Pond	1 100%	1 100%	2 100%
Stream	3 100%	4 100%	7 100%
Rainwater	0 0%	2 100%	2 100%
Other	NT	NT	NT
Total	43 100%	65 98%	108 99%

*NT – Not Tested

When grouped by type of water source and season collected the dichotomous comparisons of results are well synchronized with the exception of samples collected from rainwater sources and samples collected during the fall (Tables 8 and 9). This may be due to the fact that some of the rainwater sources were treated whereas others were untreated. Treated rainwater sources were exposed to UV sterilization, filtration, or chemical disinfection that possibly eliminated any *E. coli* that may have been present. As a result, treated rainwater sources were more likely to be negative. There is no unison seen in the dichotomous comparison of the results for the fall season due to the majority of rainwater samples being collected during fall months. Further exploration of this phenomenon may be examined in future studies.

Table 8. Type of Source and Dichotomous Comparison of Total Membrane Filtration Presumptive *E. coli* Results

Type of Source	Membrane Filtration		Total
	***E. coli* Negative**	***E. coli* Positive**	
Creek	0 0%	83 100%	83 100%
Lake	0 0%	86 100%	86 100%
Other	0 0%	4 100%	4 100%
Pond	0 0%	9 100%	9 100%
Rainwater	29 45.3%	35 54.7%	64 100%
Stream	0 0%	24 100%	24 100%
Total	29 10.7%	241 89.3%	270 100%

Table 9. Season and Dichotomous Comparison of Total Compartment Bag Test and Membrane Filtration Results

Season	Compartment Bag Test			Membrane Filtration		
	***E. coli* Negative**	***E. coli* Positive**	**Total**	***E. coli* Negative**	***E. coli* Positive**	**Total**
Fall	32 34.0%	62 66.0%	94 100%	25 26.6%	69 73.4%	94 100%
Spring	3 4.69%	61 95.3%	64 100%	0 0%	65 100%	65 100%
Summer	2 4.35%	44 95.7%	46 100%	2 4.35%	44 95.7%	46 100%
Winter	3 4.69%	61 95.3%	64 100%	2 3.08%	63 96.9%	65 100%
Total	40 14.9%	228 85.1%	268 100%	29 10.7%	241 89.3%	270 100%

DISCUSSION

The compartment bag test is a small, field portable test that performs just as proficiently as membrane filtration. The objective of our research was to perform a rigorous evaluation of the compartment bag test against the standard method 1604, membrane filtration on mI agar for the detection of *E. coli* in water. The reliability of this test is important in being able to provide a simple, reliable, low cost water quality test for use in limited resource settings.

Our evaluation indicates that the compartment bag test had high specificity (96.6%) and positive predictive value (>97%), but sensitivity (94.9%) and negative predictive value were lower (~70%). When tested against membrane filtration with mI agar, the compartment bag test was able to correctly detect 94.9% of all *E. coli* positive results, and 96.6% of all *E. coli* negative results. As a result of producing few false positives (0.45%), a positive result observed with the compartment bag test is a good predictor of the presence of *E. coli* (PPV = 99.6%). However, the compartment bag test produced a moderate number of false negatives (30%), suggesting that a negative result does not always assure the absence of *E. coli* (NPV = 70%). These results were comparable to those found by McMahan et al. with sensitivity, specificity, NPV, and

PPV determined to be 73%, 100%, 68%, and 100%, respectively for the compartment bag test.

When compared using a two sample t-test and Wilcoxon Rank Sum test for paired samples, we found statistically significant differences in concentrations for the compartment bag test compared to membrane filtration with mI agar. However, this may be due to the relatively limited most probable number combinations available in a five compartment bag test (compared to the Poisson distribution of membrane filtration). Statistically significant correlation of *E. coli* concentrations for the two methods illustrates that the quantification of *E. coli* was analogous for both methods. This suggests that the compartment bag test is comparable to membrane filtration with mI agar for the quantification of *E. coli*.

Based upon the categories of *E. coli* concentrations as specified by the World Health Organization, the compartment bag test and membrane filtration methods were in agreement when matched in each corresponding category. Results indicated that 70%, 71.4%, 73.5%, and 92.8% of *E. coli* concentrations <1, 1-9, 10-99, and ≥100 MPN/100 mL or CFU/100 mL, respectively were in agreement for the two methods. The compartment bag test is able to effectively indicate fecal contamination in water samples, thereby predicting the level of waterborne disease risk.

The unparalleled dichotomous comparison of results produced by the rainwater samples was reflected in the dichotomous comparison of results produced by water samples collected during the fall season. Treated and untreated rainwater samples were grouped together as one type of source, and therefore may have contributed to this observance. However, further investigation is needed.

Current water quality testing methods for detection and quantification of fecal bacteria can be complex and technically demanding (Boubetra et al., 2011). They require extraneous laboratory equipment, and are difficult to perform in developing countries and during natural disasters. In addition, other resources including a cold chain, electricity, and incubators are too expensive and unpractical for use in resource limited settings (Brown et al., 2011). These present immeasurable obstacles for developing countries (Chuang, Trottier, & Murcott, 2011). The compartment bag test is small and field portable which is beneficial in situations where access to laboratory equipment is not feasible. It can be used in settings where electricity may not readily be available with the utilization of ambient temperature incubation. Despite the fact that the water samples were processed in a laboratory setting for this analysis, our results demonstrate that, the compartment bag test is capable of performing efficiently in remote settings where resources are limited, such as in developing countries.

There are possible sources of variation which include color interpretation and variation in color production. In cases of indeterminate results, there may be a small or

light production of color that may only be visible with close examination, and observers may interpret color differently. There are a small number of likely positive bag compartment combinations for the compart bag test, and as a result only a small number of most probable number values (McMahan et al.).

Differences in *E. coli* concentrations were observed with samples collected from rainwater sources when tested with the compartment bag test and membrane filtration. For example, with membrane filtration on mI agar higher concentrations of *E. coli* were seen than with the compartment bag test. In some cases there were wide variations in the concentrations of *E. coli* detected by the two methods. These findings suggest the need for further investigation into why these differences were observed. One way we might do this would be to increase the number of rainwater samples collected throughout all of the seasons.

With the immediate need of a low-cost water quality test in developing countries where diarrheal disease is endemic, this test is more economically feasible to test drinking water without trained laboratory personnel. The test is rapid and simple to perform. We propose that our results suggest that the compartment bag test performs just as efficiently as the standard method, membrane filtration for the detection of *E. coli* in drinking water thereby predicting the risk of waterborne disease. In developing countries where resources for water quality testing are limited, this valuable test can aid in the prevention of diarrheal disease and help reduce the burden of disease. The compartment

bag test has the potential of being employed in monitoring activities for microbial water quality when there are excessive microbial levels detected in drinking water, and used to support water safety plans.

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