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Evaluation of the compartment bag test for the detection of *Escherichia coli* in water

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ABSTRACT

Aims: Annually, more than 2 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 *Escherichia coli* and other fecal bacteria 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 of 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. Samples were 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.

Conclusions: 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, affordable drinking water quality test where other microbial water quality testing resources are not readily available.

1. 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, making it one of the leading causes of preventable deaths worldwide and the second leading cause of mortality and morbidity in children under the age of 5 years (Liu et al., 2012). One of the major causes of the more than 2 million deaths annually 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 and Bartram, 2001). Contaminated drinking water may contain unsafe levels of microorganisms that pose a risk to human health (JMP, 2010). Fecal (also called thermotolerant) 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 bacteria in drinking water; however they can be expensive, complex, and time consuming (Boubetra et al., 2011). Most of these tests require trained laboratory personnel and a laboratory setting that may not be available in remote areas or those with limited resources (NRC, 2004). In particular, the lack of access to microbial water analysis kits or laboratories is an issue for many communities in the developing world (Sundram et al., 2000).

There is a need for simple, reliable, low-cost microbial tests that will be readily available to people and institutions in developing countries with limited access to water, sanitation, and hygiene in order to provide data that facilitate the prevention of waterborne diarrheal disease (McMahan et al., 2011). To meet this testing need, the compartment bag test, developed by researchers at the University of North Carolina at Chapel Hill, is a self-contained, portable, less expensive and simple to use test to detect and quantify the presence of *E. coli* in drinking water based upon the most probable number principle of quantification. This test can be used without extensive laboratory equipment that is normally needed for standard tests for drinking water quality. The

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purpose of this study was to evaluate the compartment bag test for the detection and quantification of *E. coli* in water against a standard method, membrane filtration using mI agar, for various natural water sources in Atlanta, GA.

2. 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. Each sample was labeled with the location of the water source and date of collection, stored at 2–8 °C, and processed within 24 h of collection. A total of 270 samples were processed and tested in the School of Public Health Laboratory at Georgia State University using the compartment bag test and the conventional membrane filtration method.

2.1. 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* and coliforms, respectively following standard method 1604 (EPA, 2002). After applying the membranes of filtered water to the agar medium, the plates were inverted and incubated for 18–24 h at 35 °C. *E. coli* colonies were quantified and reported as colony-forming units (CFU) per 100 ml (EPA, 2002).

2.2. 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 (HiMedia Laboratories, Mumbai, India) in the form of a medium bud in a sterile 120 ml vessel. Water samples of < 100 ml were brought to a 100 ml volume with sterile reagent water prior to adding the medium. The water was allowed to mix with the reagent medium bud for 20 min for medium dissolution prior to pouring into the compartment bag. The compartment bag consisted of a clear plastic bag with five internal compartments with individual volumes of 1, 3, 10, 30, and 56 ml each (Nasco, Salida, California). After 20 min, the water sample was poured into the compartment bag (typically into the largest compartment first). Then the water sample was manually distributed into each compartment by gently squeezing the bag exterior to ensure that each sample volume was filled to the set mark (Fig. 1). Each bag was then sealed using a two-piece plastic clip to isolate each compartment, and incubated for 18–24 h at 35 °C. The clip was placed across the bag above the sample levels so that each compartment was isolated from one another. Additional instructions regarding the performance of the compartment bag test can be found on the company's website (Aquagenx, 2013) (http://www.aquagenx.com/wp-content/uploads/2013/12/Aquagenx-CBT-Instructions-v3.pdf).

After incubation, each compartment of the bag was scored as positive or negative for the presence of *E. coli*. Concentrations of *E. coli* were determined using the observed positive and negative compartment bag results which correspond to the 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 outcome is based upon the principle that most *E. coli* strains produce β-glucuronidase (Watkins et al., 1988). Indeterminate results were noted and re-evaluated.

2.3. 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 agar plates were streaked for isolation successively to Bio-Rad RAPID'*E.coli* 2™ agar and then trypticase soy agar (TSA) to obtain pure colony isolates for subsequent organism identification of isolates from 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 h at 44.5 °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 h at 44.5 °C to ensure a pure culture. Colonies with the typical appearance of *E. coli* were then streaked onto TSA and incubated for 18–24 h at 35 °C. Pure isolated colonies from the TSA plates were added to individual 1 ml aliquots of TSB with 20% glycerol 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*.

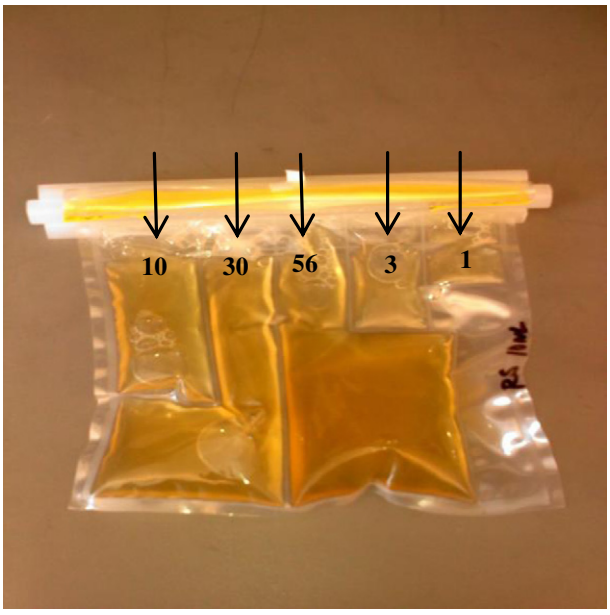

Fig. 1. Compartment bag test with sample prior to incubation. Numbers are compartment volumes in milliliters.

2.4. Data analysis

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*. The analysis included correlations between estimates for *E. coli* within the decimal categories commonly used to indicate ranges of fecal contamination. Spearman's rank correlation coefficient was used to measure how closely the *E. coli* concentrations for the two methods compared. The compartment bag test and the 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*.

Parametric and non-parametric statistical tests were used to compare results where data were and were not normally distributed,