A PERFORMANCE EVALUATION OF THE LIFESTRAW: A PERSONAL POINT OF USE WATER PURIFIER FOR THE DEVELOPING WORLD

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ABSTRACT

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A Performance Evaluation of the LifeStraw: a Personal Point-of-Use Water Purifier for the Developing World

(Under the direction of Mark D. Sobsey, Ph.D.)

18% of people worldwide have no access to safe drinking water. Many household water purifiers have been documented to improve water quality and reduce diarrheal disease. One of these technologies is the LifeStraw, a low-cost, portable, point-of-use water purifier. The LifeStraw has been used worldwide to date, however, there is not yet conclusive research about the performance of the LifeStraws ability to improve drinking water or reduce diarrheal disease burden. The purpose of this research was three-fold: to examine the microbiological capability selected LifeStraw models, to assess their life span in regards to clogging, and to ensure that disinfectant concentrations present in the effluent were below target levels. LifeStraw models tested achieved reductions of bacteria above the target of 99.9999%. Evidence suggests only moderate reductions of viruses, 90-99%. Results from this research suggest that the LifeStraw may be an effective way to improve water quality and reduce diarrheal disease from waterborne, bacterial and viral pathogens.

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Many Thanks.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ASM	American Society of Microbiology
ATCC	American Type Culture Collection
BEA	Bile Esculin Azide
BMDL	Below Minimum Detection Limit
CDC	Center for Disease Control
CFU	Colony Forming Unit
DAL	Double Agar Layer
DTW	Dechlorinated Tap Water
EPA	Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
HHWT	Household Water Treatment
ID	Inner Diameter
IWA	International Water Association
MCL	Maximum Contaminant Level
NTU	Nephelometric Turbidity Unit
NRC	National Research Council
NSF	National Science Foundation
OECD	Organization for Economic Co-operation and Development
PBS	Phosphate Buffered Saline
PFU	Plaque Forming Units
POU	Point of Use
ppb	parts per billion
SAL	Single Agar Layer
SS	Pasteurized Settled Sewage
SWS	Safe Water System

TSB	Tryptic Soy Broth
UV	Ultra Violet
VF	Vestergaard Frandsen

INTRODUCTION

Nearly 1.1 billion people around the world lack access to improved drinking water sources, and 2.2 million die from basic hygiene related disease (WHO, 2007). The majority of these deaths are wholly preventable through effective improvements in water, sanitation and hygiene. Point-of-use (POU) water treatment is by no means the silver bullet for eliminating the waterborne disease risks and burdens of these 1.1 billion people. POU technologies such as the LifeStraw are key components to reducing the disease burden in the short term. In some cases, they can provide a daily source of affordable, less contaminated or uncontaminated drinking water.

In most industrialized countries, waterborne disease has been of modest concern since the end of the sanitary reform movement in the early twentieth century (Andrews, 2006). Pathogens that have been of recent concern in the industrialized world are those that continue to evade treatment processes like chlorination and filtration. Enteric viruses and protozoan parasites (e g *Giardia lambia* and *Cryptosporidium parvum*) are of continued concern in the developed world, whereas bacterial pathogens like *Vibrio cholerae* and *Salmonella typhi* are sensitive to disinfection and over the past century, have seen a steady decline as disease agents (OECD, 2007). However, in much of the developing world bacterial pathogens continue to represent a large portion of the infectious disease burden. Most of these bacterial pathogens are waterborne (pathogen transmission through ingestion of contaminated water) or water-washed (transmission favored by inadequate sanitation or hygiene practices) (White,

1972). Common pathogens representing the two categories of transmission include *Vibrio cholerae, Shigella, Salmonella typhi, Campylobacter jejuni,* various pathogenic strains of *Escherichia coli* and *Yersinia* species (Schlosser, Robert, Bourderioux, Rey, & de Roubin, 2001).

Concern about waterborne disease in areas without established water treatment infrastructure has led to the development of small-scale, water treatment devices sized for household use that are affordable for the individual or family. The LifeStraw is one of the newest and most promising of the individual sized POU water treatment devices that can be used daily or for temporary use in emergencies.

1.1 Aims

1.1.1 Aim one

To evaluate the microbial effectiveness of candidate models of LifeStraws in reducing waterborne bacteria, viruses and protozoan parasites under laboratory conditions designed to mimic natural drinking water quality conditions typical of those found in developing countries. The ability of the LifeStraw to meet US Environmental Protection Agency (EPA) and National Science Foundation-International (NSF) regulatory standards and guidelines for the reduction of three major classes of microbes (i.e. bacteria, viruses and parasites) is a crucial gauge of its effectiveness as a POU water treatment device.

Previous laboratory studies of the LifeStraw, as well as a basic understanding of the treatment components within the device, influenced the selection of test microbes. Test microbes varied among our experiments; but each experiment included microbes representing each (see Table 1). Bacteria, viruses, and protozoa are not the only three categories of waterborne pathogens; however, parasitic helminthes were not included in the

study. The design of the treatment device allows for complete exclusion of both the ova and adult worms in the effluent water by physical removal at the pre-filter.

Table 1: Test microbes and Lifestraw model division

Models	Bacteria (gram +)	Bacteria (gram -)	Virus	Protozoa
C1-C5	*E. faecalis*	none	MS2 coliphage	*C. parvum*
NVO and YAO	*E. faecalis*, *C. perfringens*	*E. coli* KO11, *V. cholera*, *C. jejuni*, *S. typhimurium* WG-45	MS2 coliphage	*C. parvum*
L	*E. faecalis*	*E. coli* B	MS2 coliphage	*C. parvum*
F	*E. faecalis*	*S. typhimurium*, *E. coli* B	MS2, Poliovirus	*C. parvum*, *G. lambia*

1.1.2 Aim two

The ability of the LifeStraw to reduce pathogens in water is only important in the context of a physically well-functioning device. Without the ability to pass a sufficient amount of water with minimal effort, the LifeStraw is of little benefit to its user, regardless of its efficacy at microbial reductions. World Health Organization (WHO) guidelines recommend that two liters of water per day should be the universal minimum daily requirement for drinking water (WHO, 2007).

The goal set forth by Vestergaard-Frandsen, the makers of the LifeStraw, is for each device to be able to meet or exceed the WHO minimum daily water volume requirement for approximately one year (Frauchiger, 2007). From these guidelines, the second aim of the laboratory challenge is, specifically, to challenge the devices to pass at least 700 L of water without clogging. When clogging occurs to the point where (1) the average person would not be able to efficiently draw water through, or (2) the treatment mechanisms deteriorate to the point that the LifeStraw is no longer effective at microbial reduction, the LifeStraw would be considered unsatisfactory in performance. LifeStraws have the ability to be backwashed.

Backwashing temporarily improves ease of water flow through the device. With extensive use, or when used with high turbidity influent water, backwashing can become less effective. The goal is to determine if the target volume of 700 liters could be filtered with typical periodic backwashing procedures as used by the consumer.

1.1.3 Aim three

The third objective for testing is to track the concentrations of iodine and silver in the effluent water from the treatment processes within the LifeStraw. In many water treatment scenarios chemical surplus or residual in effluent water is intended to continue or maintain microbial reductions during storage. However, with a POU treatment device like the LifeStraw, the effluent water is not storable, but is immediately ingested. The EPA sets standards and WHO sets guidelines for a number of chemical contaminants in water. Goals for the third objective were set using EPA secondary standard for silver (≤100 ppb), and WHO's recommendation for healthy iodine levels in drinking water (≤0.1 mg/L/day) (EPA, 2006; WHO, 2006). In the LifeStraw, iodine potentially originates from the iodinated anion exchange resin, while the silver potentially originates from a silver impregnated carbon block filter element. There are no other known chemical disinfectants or disinfectant byproducts produced or released by the LifeStraw.

1.2 Experimental overview

The LifeStraw project consists of four consecutive challenge experiments. All aspects of the project were conducted under laboratory conditions. Figure 1 illustrates the structure of the experimental design, including important variables. Each of the four experiments took approximately two months each, with three months of preparation time for system setup and to establish methods and microbe stocks for microbial analysis. Two key aspects of the

series of experiments were aging, passing water through LifeStraws that has no added test microbes, and challenging, periodically passing water through LifeStraws that contained known amounts of test microbes.

Figure 1: Plan of sequential LifeStraw experiments, including key variables

Series	NVOYAO		C	L		F	
Model	NVO	YAO	C1 C2 C3 C4 C5	L1	L2	L2	F
# of Replicates	5	5	1 of each	3	4	5	5
Water type	SS	SS	DTW	2-DTW 1-SS	3DTW 1-SS	SS	SS
# of Microbes used	9	9	3	4	4	4	4

Aging water representing one of two types of water quality ("clean" and "dirty") was pumped to versions of three different LifeStraw models in an attempt to assess two variables over time; (1) susceptibility to clogging and (2) extent of leaching of chemical disinfectant. "Clean" water was simulated by using dechlorinated tap water, while "dirty" water was simulated by the use of dechlorinated tap water supplemented with 1% pasteurized settled sewage.

LifeStraws were periodically assessed throughout aging for changes in flow rates and chemical concentrations of residual disinfectants in effluent water. When the flow rate for an

individual device consistently fell below 150 ml/min and could not be restored by backwashing, analysis was discontinued. Challenge procedures using microbe- seeded water occurred at regular intervals throughout the aging schedule, and were designed to assess the ability of the straws to consistently reduce microbial concentrations throughout the straw lifespan. Influent water was seeded with known concentrations of a variety of indicator and pathogenic microbes. This water was pumped through the LifeStraws, the effluent water was collected and analysis was then performed to determine the microbial concentrations in the effluent water as well as the influent challenge water. The difference in microbial concentration between influent and effluent water represented the disinfection or microbial reduction ability of each respective LifeStraw. Chemical analyses for residual concentrations of iodine/iodide and silver were also a part of the challenge procedure. Aging procedures continued until a total of 700 L of water had passed through each straw. Challenges with microbe-seeded water occurred at increments of approximately every 100 L of aging water throughout the duration of the aging procedure.

Eleven microbes were used in challenge tests throughout four successive experiments. Both indicator microbes, as well as pathogens, were tested. Only the results for indicator microbes are presented in this report. The results for pathogens are presented in a separate report by Masters Candidate, Erin Printy. Figure 2 lists the indicator and pathogenic microbes used.

Figure 2: Indicator and pathogenic test microbes

Indicator organisms	Enteric pathogens
• *Escherichia coli* B	• *Campylobacter jejuni*
• *Escherichia coli* KO11	• *Cryptosporidium parvum*
• *Clostridium perfringens*	• *Giardia lambia*
• *Enterococcus faecalis*	• Poliovirus type 1, Strain LSc
• MS2 coliphage	• *Vibrio cholera*
	• *Salmonella typhimurium*

LITERATURE REVIEW

2.1 Infectious disease and the burden of diarrheal disease

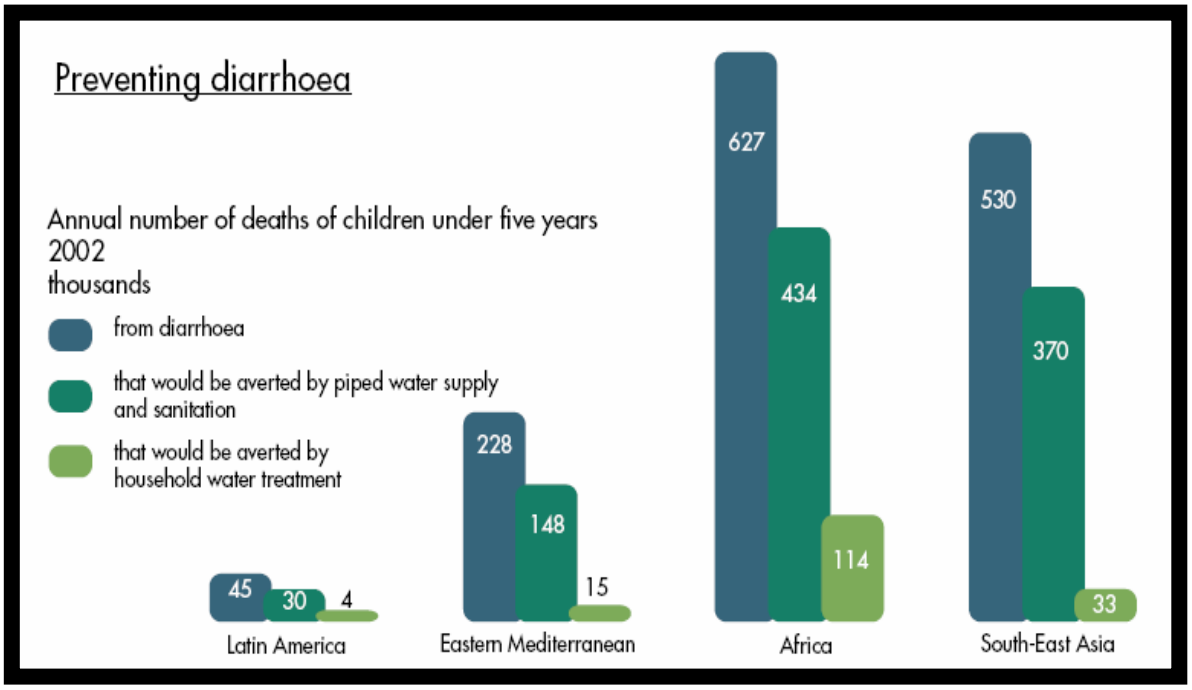
Throughout history, infectious diseases have undoubtedly been the single largest contributor to human morbidity and mortality. At least 13 million people die each year from preventable and often times curable infectious disease. Half of these deaths are children and nearly all occur in developing countries (Esrey, Feachem, & Hughes, 1985). In the past century we have seen the burden of infectious disease plummet in what are now the developed nations of the world (Esrey et al., 1985). In fact, the 20th century will likely be remembered for its leaps in technological advances, the reform of sanitation infrastructure in developed countries, the acceleration of global markets and communication, and the urbanization of many nations.

Despite these changes, the majority of the world still battles with communicable disease; over a billion people lack access to adequate water and over twice that number are without adequate sanitation (WHO, 2001). There is no single answer to these enormous disparities. Strong political leadership, improvements in water and sanitation infrastructure, sustainable development of global markets, innovative ideas and technologies, and community-level solutions are all required to make tangible, lasting improvements.

Diarrheal disease is an important contributor to the global disease burden. Figure 3 illustrates the global burden of diarrheal disease by region. With 4 billion cases of diarrhea annually, 88% of which are directly attributable to consumption of unsafe water or

inadequate sanitation, over half the population of the world is affected. (WHO, 2006) The burden of diarrheal disease is particularly culpable because it is preventable through improved access to safe water and sanitation, and hygiene education. (Kosek, Bern, & Guerrant, 2003). The WHO estimates that 94% of diarrheal disease is preventable through changes in environment (WHO, 2006).

Figure 3: Global burden of preventable diarrhea



(WHO, 2007)

Diarrheal diseases kill an estimated 1.8 million people each year (Kosek et al., 2003). In developing countries diarrhea accounts for 17% of deaths of children under five (Eisenberg, Scott, & Porco, 2007). For the 1.1 billion people who lack access to improved water supplies, and many more with microbially contaminated water, diarrheal disease is highly endemic. (Clasen, Schmidt, Rabie, Roberts, & Cairncross, 2007) Studies have shown that water, sanitation, and hygiene interventions, as well as their combination, are effective at

reducing diarrheal illness, and water quality interventions such as POU treatment technologies have been more effective than previously thought. (Fewtrell & Colford, 2005)

2.2 Household water treatment

Conventional piped water systems using effective treatment to deliver safe water to households may be decades away in much of the developing world. This leaves the majority of the poorest people in the world left with the task of collecting water outside the home, then treating and storing it themselves (Sobsey 2002). Taking steps to remove or inactivate enteric pathogens in drinking water immediately before consumption has been shown to be effective in reducing diarrheal disease (M.D. Sobsey, 2002). This somewhat intuitive finding is significant because many households in less developed countries do not have individual connections to treated, piped water, or 24–hour access to water. Such households typically store water in the home, and this water is vulnerable to contamination during transport and storage, even if it is free from microbial or chemical contaminants at the source (Mintz, Bartram, Lochery, & Wegelin, 2001). In cases such as these, a water quality intervention at the point-of-use should be considered for any water supply program (Fewtrell & Colford, 2005). A safe water source refers to household connection, public standpipe, borehole, protected (lined) dug well, protected spring, or rainwater collection and safe storage (Cairncross & Valdmanis, 2006).

Several peer-reviewed studies support Fewtrell's findings, reporting rate ratios that suggest household based interventions are more effective at preventing diarrhea than water source based interventions. (Clasen et al., 2007; Mintz et al., 2001; M.D. Sobsey, 2002)

In response to the relentless burden from waterborne diseases worldwide, new strategies for safe water have been identified as a key to improving public health in

developing countries. Treating drinking water at the household level to reduce the ingestion of pathogenic microbes is one suggested strategy (M.D. Sobsey, 2002). Devices that can be used to either treat water or prevent contamination of stored water in the home are referred to as POU technologies. These technologies provide a means for individuals and families to reduce microbial and chemical contaminants in drinking water at the household level. POU technologies have the potential to fill the service gap where piped water systems are not yet feasible, potentially resulting in positive health impacts in developing countries (Sobsey 2006). Two recent meta-analyses of field trials have suggested that household-based water quality interventions such as appropriate treatment and safe storage are effective in reducing diarrheal disease (Clasen et al., 2007; Fewtrell & Colford, 2005; M.D. Sobsey, 2002).

A variety of technologies for POU water treatment exist; some are based in historical water treatment techniques, however, there is new research that has found effective reduction of waterborne pathogens and diarrheal disease using innovative POU technologies like the Biosand filter and simple ceramic "candle" filters (Lantagne, 2007). Figure 4 describes a number of the most common POU water treatment methods.

Figure 4: common point-of-use treatment methods

Boiling	1. Simple method for the inactivation of viral, parasitic, and bacterial pathogens. 2. Often economically and environmentally unsustainable. 3. Provides no residual protection 4. There is a significant risk of scalding among infants. (Mintz et al., 2001)
Solar Disinfection	1. Uses the synergy of solar UV and heat 2. Simple, inexpensive, does not affect taste 3. Ineffective with turbid water 4. Not good for large volumes (Mintz et al., 2001)
Chlorination	1. Sodium hypochlorite, has proven the safest, most effective, and least expensive chemical disinfectantfor point-of-use treatment. 2. It can be produced onsite or created onsite through electrolysis 3. Relatively ineffective against parasites and viruses 4. The taste and odor of chlorinated water can reduce use (Mintz et al., 2001)
Blends	1. **PUR sachet:** a packet containing powdered ferrous sulfate (a flocculant) and calcium hypochlorite (a disinfectant). Very effective even with turbid water

	2. **One Drop:** an aqueous solution of ionic minerals, including silver, gold, aluminum, zinc, and copper. It is a simple, low-cost, effective solution. (CDC, August 2005), (Murphy, 2007)
Filtration	1. Many types available for POU treatment • Granular media: Bio-sand, slow sand • Vegetable and animal derived depth filters • Membrane filters: paper, cloth, plastic • Porous cast filters: ceramic pots • Septum and body feed filters 2. Filtration alone, at a household level, has not proved effective for viruses and acceptable reductions of bacteria. (M.D. Sobsey, 2002)
Ultraviolet	1. Works very well on all waterborne pathogens in combination in parallel with a turbidity reducing treatment like coagulation/flocculation or filtration. 2. No odor or taste problems 3. Requires significant energy input: batteries or electricity (M.D. Sobsey, 2002)

2.3 LifeStraw design and treatment processes

In 2005, Vestergaard Frandsen developed the first model of the LifeStraw. The Vestergaard Frandsen (VF) Company was founded by Kaj Vestergaard Frandsen in 1957 in Kolding, Denmark. The primary achievements of VF are as a designer and producer of insecticidal polyester bed nets to prevent the spread of malaria (Frandsen, 2007).

LifeStraw is a personal POU filtration device designed for daily use to decontaminate relatively small volumes of drinking water (2L per day) (Frauchiger, 2007). In response to a growing concern for microbial contamination of drinking water used by children, the LifeStraw is designed for children both in its ease of use and its portability. The LifeStraw is low-cost, low-tech, easy to transport, and requires no electric or mechanical power input. These qualities make it a reasonable means of reducing waterborne microorganisms in drinking water in large-scale disasters like the Indian Ocean tsunami of 2005. However, the LifeStraw has drawbacks: it cannot be used for large volumes of water, it is not able to produce water to be stored, and residual iodine can leave an unpleasant taste in the effluent water.

The standard model LifeStraw is 31 cm long and 2.9 cm in diameter with a dry weight of 140 grams. The outer shell of the LifeStraw is made of high impact polystyrene. VF has designed the LifeStraw to be effective for up to one year of use based on consumption of 2 L per day from the straw. There are three compartments within the straw that aid in microbial reduction. At the base of the straw there is a 15 micron (minimum) plastic mesh screen designed to filter relatively large particle contaminants and organic matter. After passing the screen, water enters a compartment of halogenated ion exchange resin that elutes an active halogen, most often free iodine.

Iodine is a halogen with strong oxidant chemical properties, giving it useful biocidal effects. The active disinfectant forms of iodine are elemental iodine and hypoiodous acid. Iodide is not a viable disinfectant. Water disinfection with iodine is a first-order chemical reaction. The disinfection of different classes of microorganisms by iodine vary in effectiveness. Bacteria are sensitive to iodine, viruses are intermediate, and protozoan cysts are more resistant. Doses of iodine below 1 mg/L are effective for bacteria within minutes; however, at the same concentration, it would take many hours to kill protozoan parasites like *Giardia lambia* and *Cryptosporidium parvum*. Recommended levels of iodine for point-of-use water disinfection in unmonitored field situations are relatively high to allow for unanticipated reactions with organic contaminants and to allow for a short contact time for effective disinfection. (Backer, 2000)

A section of silver impregnated granular activated carbon (GAC) provides a dual-function; first it acts as a means of adsorbing free iodine eluted from the ion exchange resin, and the impregnated silver provides a second stage of disinfection as well as preventing the

growth of biofilm on the GAC. The duo of active disinfectants is intended to effectively inactivate waterborne bacteria and viruses. (Frauchiger, 2007)

2.3.1 Iodine and halogenated resins

The initial compartment of the LifeStraw contains an iodinated resin in the form of small polymer beads. The resin beads provide a cationic surface to which elemental iodine is attached. As water passes through the resin beads the iodine is released into the water through the process of ion exchange. Ion exchange happens when the negatively charged particles in the water surrounding the resin displace the iodine leaving free iodine to attach to the cell wall or membrane of microbes in the water. The result of ion exchange is an increased level of iodine in the contaminated water to provide a considerable biocidal effect (Edison, 2002). Figure 5 compares the advantages and the disadvantages of using iodinated resin in POU water treatment devices.

Figure 5: Advantages and disadvantages to the use of iodinated resins in point-of-use water treatment

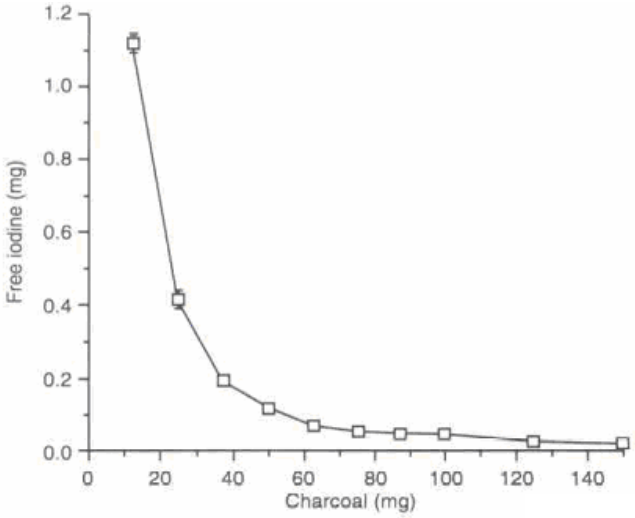
Advantages	Disadvantages
1. Effective against parasites, bacteria, and viruses	1. Does not work well with highly turbid water, turbidity should be less than 1 NTU
2. Low maintenance, no needed energy source	2. High pH and temperature can cause releases Iodine at harmful levels
3. Iodine is not prone to form harmful byproducts	3. Influent water with a high existing halogen demand can quench free Iodine decreasing biocidal effects
4. Contact time with the resin beads required for microbial reductions is relatively short	

(Edison, 2002)

2.3.2 Silver impregnated carbon block

Activated carbon has been used for decades in municipal drinking water treatment to remove odors and tastes from suspended organic matter. The primary purpose for the use of a carbon block with the LifeStraw is to adsorb free iodine from the anion exchange resin.

(Sahal, 1999) Figure 6 illustrates the capacity for carbon to bind free iodine. However, carbon blocks have been shown to develop biofilms on their outer surface. Insoluble organic compounds on the outer walls of the carbon block are a source of nutrients for bacteria found in water. When the carbon block sits in stagnant water, bacteria thrive on the nutrients from the ash and are flushed at relatively high concentrations during the next use (Seelig, 1992). Silver impregnated carbon uses the biocidial effects of silver to prevent the growth of biofilm on the carbon block. The process begins as microbes are adsorbed into the impregnated carbon block. Here it comes in contact with silver ions and the sulphurhydryl group within the cells react producing a silver-sulphur molecule. This silver-sulphur combination immobilizes the respiratory activity of the cell by preventing the transfer of protons (Bayati, 1997). Silver is regulated as a pesticide under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and its use is monitored by the EPA. The EPA has established a Secondary Maximum Contaminant Level (MCL) of 0.1 milligrams of silver per liter of water or 100 parts per billion (ppb). (EPA, 2006)



(Each data point represents the average of three observations)

2.4 Indicators for waterborne pathogens

2.4.1 Background

The use of bacterial indicators for water quality measures dates back to 1880 when Von Fritsch described *Klebsiella pneumoniae* and *Klebsiella rhinoscleromatis* as micro-organisms routinely found in human feces. (Geldreich, 1969) Soon after Fritsch's discovery, Escherich described *Bacillus coli*, later named *Escherichia coli*, from the feces of breast-fed infants (Geldreich, 1969).

By the 1890s, it was decided that direct pathogen detection would be too complex for public health protection because 1) there are too many pathogens, 2) they are present in small concentrations, and 3) methods for their detection are delicate and expensive. Public health officials decided that monitoring would be conducted to detect fecal pollution rather than individual pathogens. (Leclerc, Edberg, Pierzo, & Delattre, 2000)

In the US, the original purpose for the use indicators was for the detection of contaminated drinking water. However, as the nation developed, indicators were used primarily in the detection of human fecal contamination of ambient and recreational waters. (NRC, 2004) In the developing world, indicator microbes are used primarily for the detection of fecal contamination of drinking water because of the lack of effective large scale water treatment and wastewater infrastructure (Ashbolt, 2001).

2.4.2 Purpose and use

The primary motivation for the development and use of microbial indicators is in replacement of direct pathogen detection. Pathogen detection is considerably more expensive, technically difficult, and can lend to uncertainty and inaccuracy regarding the extent of contamination (NRC, 2004). Microbial indicators are most commonly used either

to identify environmental contamination or to evaluate the effectiveness of a microbial reducing technology. The latter use is especially important for the development of effective technologies to be used in a developing world setting because of the high burden of disease sourced from fecal contamination of drinking water. This high occurrence of fecal contamination of water leading to waterborne disease paired with the consumption of untreated or ineffectively treated drinking water necessitate a simple, accurate, low-cost means of health risk assessment. (Moe, 1991)

2.4.3 Defining characteristics

Microbial indicators are used in three distinct practices as shown in Figure 7. The categories are not mutually exclusive; therefore a specific indicator could be used in any of the three use categories.

Figure 7: Categories of indicator organisms

Process Indicators	Used in determining the efficacy of a process. i.e. coliforms for iodine
Fecal Indicators	Infers the presence of fecal contamination in natural waters
Model Organisms	A species that is indicative of pathogen presence and behavior. i.e. *E. coli* as an index for *Salmonella*

Adapted from, (Ashbolt, 2001)

Microbial indicators can be an ideal solution to the need for a fast, relatively simple, and inexpensive alternative to direct pathogen detection when used properly and selected appropriately according to specific biological attributes (NRC, 2004). Figure 8 lists important biological attributes of indicators.

Figure 8: Desirable biological attributes of indicators

- Correlated to health risk
- Similar or greater survival and transport to pathogen
- Present in greater number than pathogen in the environment
- Specific to an identifiable source, e.g. human fecal matter
- Applicable to various types of water
- Does not create false positives
- Non-pathenogenic to humans

Other important attributes of a good indicator organism are the ease of use, a low cost for detection, easily quantifiable methods, precision, and oftentimes rapid results (NRC, 2004). A further criterion for a good indicator is offered in *Bonde's Critera for an Ideal Indicator* published in 1966 and illustrated in figure 9.

Figure 9: Bonde's criteria for an ideal indicator

An ideal indicator should:

1. Be present wherever the pathogens are present;
2. Be present only when the presence of pathogens is an imminent danger (i.e. they must not proliferate to any greater extent in the aqueous environment);
3. Occur in much greater numbers than the pathogens;
4. Be more resistant to disinfectants and to the aqueous environment than the pathogens;
5. grow readily on simple media;
6. Yield characteristic and simple reactions enabling as far as possible an unambiguous identification of the group
7. Be randomly distributed in the sample to be examined , or it should be possible to obtain a uniform distribution by simple homogenization procedures; and
8. Grow widely independent of other organisms present, when inculcated in artificial media (i.e., indicator bacteria should not be seriously inhibited in their growth by the presence of other bacteria).

Adapted from, (Bonde, 1966)

Best practice when using indicators is to use a "tool box" of indicators in which a diverse set of indicators and methods are matched to specific goals for water quality. Using a variety of indicator microbes can provide clues into the source and specific type of contamination. (NRC, 2004) For example, a combination of indicator bacteria could be used to differentiate between fecal contamination by livestock and that of human fecal matter as well as providing insight into the source of contamination.

There are a variety of subsets of indicator organisms contained within the classifications of either bacterial indicators or viral indicators. Two of the most common bacterial indicators are coliforms and fecal streptococci. Bacterial indicators not only serve as means of detecting pathogenic bacteria, but can also mark the presence of fecally transmitted protozoan parasites, viruses and even helminthes. (NRC, 2004)

2.4.4 Coliforms

Total coliforms can be defined as aerobic and facultatively anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that produce gas upon lactose fermentation within 48 hours at 35°C (Bitton, 2005). Fecal coliforms are the most commonly used indicator organisms,; they include all coliforms that can ferment lactose at 44.5°C. (Ashbolt, 2001) Coliforms occur in the intestines of all warm-blooded animals and are found in densities proportional to the degree of fecal contamination in polluted waters. Under the provisions of the National Primary Drinking Water Regulations, total coliforms are used as a standard for the microbial safety assessment of ambient and recreational waters throughout the US. Some of the most well known fecal coliforms are *Escherichia coli* (*E. coli*) and species within the genus *Enterobacter*. (Ashbolt, 2001)

E. coli is a thermophilic coliform producing indole from tryptophan, as well as often producing β-glucuronidase. (NRC, 2004) Of the coliforms, *E.coli* is by far the most common, comprising 96.8% of coliforms detected in a 1977 study of 28 fecal samples (Dufour, 1977). The WHO affirms *E. coli* to be the most appropriate of the coliforms to indicate fecal pollution from warm-blooded animals (Ashbolt, 2001). *E. coli* is so commonly used in part because of its ability to be easily distinguished from other indicators of fecal pollution by the absence of urease and presence of β-glucuronidase (Bitton, 2005).

2.4.5 Enterococci

Fecal streptococci are present in the feces of most warm-blooded animals. A number of species have been consistently recovered from known contaminated waters and have not been found to endure in the environment (Geldreich, 1969). Fecal streptococci are gram-positive, grow on bile-esculin agar and at 45°C, belong to the genera Enterococcus or Streptococcus; they possess the Lancefield group D antigen. Fecal streptococci are tolerant of sodium chloride and alkaline pH levels; they are also facultatively anaerobic and grow in small chains or pairs (NRC, 2004).

Enterococci are a subset of fecal streptococci often called the intestinal enterococci. Enterococcus is a genus of bacteria of the phylum Firmicutes. Members of this genus were classified as Group D Streptococcus until 1984 when genomic DNA analysis indicated that a separate genus classification was appropriate. Intestinal enterococci are valuable bacterial indicators for determining the extent of fecal contamination of natural waters. They include all fecal streptococci that grow at pH 9.6, 10° and 45°C and in 6.5% NaCl. Enterococci are also defined by their resistance to 60°C for 30 min and the ability to reduce 0.1% methylene blue. (Ashbolt, 2001) The two most commonly used Enterobacteria species used as

indicators are *E. faecalis* and *E. faecium*; which are commensal organisms in the intestines of humans. Enterococci are also facultative anaerobes. (Abbott, 2006)

Enterococcus was originally selected to be used as an indicator bacteria because, 1) it occurs in high numbers in the excreta of humans and other warm-blooded animals; 2) it is present in wastewater and other polluted waters; 3) it is absent from ecologically sound ambient waters and environments; and is persistent without multiplication in the environment. (Clesceri, 1992)

Enterococci are detectable by simple, inexpensive cultural methods that require basic laboratory facilities. Commonly used methods include membrane filtration with incubation on selective media incubation at 35–37 °C for 18-24 hours. (Clesceri, 1992)

Water quality guidelines based on bacterial density have been proposed for U.S. recreational waters. For fresh waters the guideline is 33 enterococci/100mL while for marine waters it is 35/100mL. Each guideline is based on the geometric mean of at least five samples per 30 day period during the swimming season. (EPA, 1986)

2.4.6 Bacteriophages

In the 1970's a new found awareness of the importance of enteric pathogens to water related public health led to the finding that viral contamination in drinking water could not be accurately measured by bacterial indicators such as coliforms (NRC, 2004). Since direct viral pathogen detection was complicated because of the large number of enteric viruses present in contaminated water and the difficulty of detection at low concentrations, scientific focus turned to the use of viral indicators. (Leclerc et al., 2000) Three groups of bacteriophages have been identified as suitable indicator organisms.

Somatic coliphages have been shown to correlate the best of the three types of bacteriophages with enteric viruses. (Geldenhuys, 1989) Starting in 1948 Guelin was the first to use coliphages as indicators of fecal contamination. Twenty years later, a number of studies explored the use of coliphages as indicators of enteric viruses. Somatic coliphages can infect a number of species of the genus enterobacteriaceae, however, *E. coli* is the primary host. Coilphages make good indicators because they are found in higher numbers than enteric viruses in wastewater, and they are easy and quickly detected. (Bitton, 2005) Somatic coliphages are characterized by their ability to attach to the cell membrane of the host. Once attached, the phage will send nucleic acid through the outer membrane, into the cell to alter the reproductive organs of the bacterial cell to create more viruses.

Male-specific RNA phages are single stranded phages, belonging to the family Leviviridae that attach to host cells at the male sex pili. FRNA phages are not considered as fecal indicators because they are not consistently found in human fecal matter and they do not have a direct relationship with the level of fecal contamination. (Bitton, 2005)

Phages infecting *Bacteroides fragilis* have been detected at low concentrations in human feces and not at all in animal feces or in pristine environments. Phages of *B. fragilis* do not multiply in environmental samples, and are more resistant to chlorine than bacterial indicators. Persistence and reductions of *B. fragilis* phages are similar to enteroviruses and rotaviruses making them suitable as indicators of human fecal pollution. (Bitton, 2005)

There has been controversy over the use of phages as indicators of fecal contamination primarily because phages are in fact indicators of indicators. That is, they indicate the presence of bacteria like *E.coli* or *coliforms* that are themselves indicators of the presence of enteric pathogens. Further arguments point out that host bacteria must be in

relatively high concentrations (10^4 CFU/mL) to support successful phage replication. In both ground and surface waters with fecal contamination, environmental conditions are often not suitable to support phage replication. (Leclerc et al., 2000) However, the use of indicator phage to mimic the physiological and biological characteristics of enteric viruses has been supported and provides a useful means of testing the efficacy of treatment processes without the use of enteric viruses.

2.4.7 Clostridium perfringens spores

Clostridium perfringens (C. perfringens) is a gram positive, anaerobic, sulfite reducing bacteria known for producing extremely resistant spores. *C. perfringens* spores are resistant to UV radiation, temperature and pH extremes, chlorination, and exposure to ethanol (NRC, 2004). The Clostridium genus contains both pathogenic and indicator species; *C. perfringens* is the characteristic species of the genus and is commonly found in the intestinal flora of many warm-blooded animals including humans. (Ashbolt, 2001) *C. perfringens* is not normally found in natural waters making it a highly specific indicator for fecal contamination. Characteristics that make C. perfringens a good indicator include its resiliency in the environment, its consistent presence in sewage, and its inability to multiply in water. Detection methods for C. perfringens are relatively simple: membrane filtration and anaerobic incubation on a selective agar media. (Clesceri, 1992)

METHODOLOGY

3.1 Design of the LifeStraw feed water pumping station

The LifeStraw pumping station consisted of two manifolds, each of which housed a maximum of five LifeStraw units mounted vertically. Influent and effluent tubing on the manifolds was ¼" ID, clear silicone. Two dual purpose oil filled pumps were used to pump influent water into the LifeStraws. The pumps were manufactured by the Little Giant pump company, pump model number 2E-38N: 115V. Before influent water entered each LifeStraw the water passed through an adjustable, acrylic flow regulator of the rotameter type, which was used to maintain an influent flow rate of 150 mL/min. The rotameter type flow meters were manufactured by Key Instruments: series FR4000. Effluent water discharged from the LifeStraws through clear silicone tubing that was directed into a floor drain.

LifeStraw models

Over six months a series of four successive challenge experiments used a total of 40 LifeStraws representing thirteen model types. (Table 2) When LifeStraw models were received by UNC staff, the company description was recorded and each straw was given a series, model, and unit label (Table 2). The progression of experiments began with the NVO and YAO series, the second group tested was the C series LifeStraws, the third experiment tested the L series units, and the most recent experiment was labeled the F series. For each of the five prototypes, there were a variety of designs that varied based on a single characteristic or functional property (e.g. pre-filter pore size, iodine resin compartment size, activated

carbon-silver content, number of compartments). The variable for the NVO and YAO models was the bead size for the iodinated anion exchange resin. The F and L model variables were the pre-filter pore size. The C series consisted of five sub-model types with varying ratios of disinfectant ingredients (iodine resin and silver impregnated carbon) one replicate of each of the five sub-models were tested.

Table 2: LifeStraw Model Details

Date received	VF label	Model name	UNC unit label	# Tested	Characteristics	Notes
Jan-07	LF64 NVO	NVOYAO/NVO	NA - NE	5	not segmented size of granule	1% ss; 9 microbes
Jan-07	LF64 YAO	NVOYAO/YAO	YF - YJ	5	not segmented; size of granule	1% ss; 9 microbes
Feb-07	LF64 modC	C1	C1	3	segmented; same as YAO	2 = dtw; 1 = dtw + 3 microbes
Feb-07	LF64 modC2	C2	C2	3	segmented; ingredients ratios	2 = dtw; 1 = dtw + 3 microbes
Feb-07	LF64 modC3	C3	C3	3	segmented; ingredients ratios	2 = dtw; 1 = dtw + 3 microbes
Feb-07	LF64 modC4	C4	C4	3	segmented; ingredients ratios	2 = dtw; 1 = dtw + 3 microbes
Feb-07	LF64 modC5	C5	C5	3	segmented; ingredients ratios	2 = dtw; 1 = dtw + 3 microbes
5-Jul-07	LF07 008 A	L1	L1, L2, L10	3	6 uM prefilter	1 = dtw + 4 microbes; 1 = ss + 4 microbes
20-Jun-07	LF07 008 B	L2	L3 - L5, L7, F6 - F10	8	15 uM prefilter	3 = dtw + 4 microbes; 5= ss + 7 microbes
15-Jun-07	LF 07 101	L3	L6	1	11 uM prefilter	1 = ss + 4 microbes
15-Jun-07	LF07 103	L4	L8	1	20 uM prefilter	1 = ss + 4 microbes
15-Jun-07	LF07 104	L5	L9	1	27 uM prefilter	1 = ss + 4 microbes
Aug-07	LF64	F	F1 - F5	5	segmented; ingredients ratios	5 = 1% ss; 7 microbes

ss: pasteurized settled sewage dtw: dechlorinated tap water

3.2 Aging days

The purpose of aging was to determine the effective water volume lifespan of the LifeStraws based on performance with respect to clogging and chemical leaching. Aging water was held in two 100+ liter plastic barrels, each containing 91L of aging water. Aging water was either dechlorinated tap water or dechlorinated tap water with 1% settled sewage. The type of aging water varied throughout the experimental series and between LifeStraws. The aging water used in each set or series of experiments is indicated in Table 2.

To prepare settled sewage for experimental use, secondary effluent was collected from Chapel Hill's Mason Farm wastewater treatment plant, pasteurized by exposure to 70°C for 30 minutes, then decanted and stored at 30°C.

Presence of chlorine in dechlorinated (activated carbon-filtered) tap-water in the laboratory was tested prior to filling the tanks with aging water using the Hach total chlorine kit. Temperature was also measured in the tanks before aging began. The first water to be pumped from the aging tanks was used to backwash the LifeStraws. Backwashing was routinely performed to mimic use by consumer. To backwash, the system was reversed and aging water was pushed down the LifeStraws from the mouthpiece and out the intake tubing. During backwashing a pressure of 3 – 4 psi (0.2 – 0.275 bar) was applied to the mouthpiece of each straw for approximately 15 seconds. The pressures used to push water through the LifeStraw were selected to mimic use by the consumer. Maximum static inspiratory pressure has shown to vary with sex, age, height, and health. Research has shown that humans can create inspiratory pressures of anywhere from 50 cm of water to 125 cm of water. (Collett, 2002) The American Thoracic Society/European Respiratory Society notes that a human can create an inspiratory pressure of 100 cm H_2O (*Statement on Respiratory*

Muscle Testing, 2001). Personal communication with Dr. James Yankaskas and Dr. Robert Tarran from the Pulmonary and Critical Care Medicine Center at the University of North Carolina supported the finding that 100 cm H_2O (approximately 0.1 bar) would be a reasonable number for the LifeStraw research.

Following backwashing, LifeStraws were aged for 10 minutes (~1.5 L/LifeStraw), after which time an effluent sample was collected from each LifeStraw for chemical analyses. Total iodine (iodide + iodine) was tested using the Taylor Midget Comparator Test; the detection limit is 0.2 mg/L. If there was detectable iodine, presence of iodide was tested using the same test kit. Effluent water was also tested for presence of silver using the Hach Rapid Silver test kit, the lower detection limit was 5 ppb. As aging resumed, flow rates were monitored and adjusted as necessary to maintain 150 ml/min until the remainder of the aging water for an increment of the ultimate total flow had passed through the LifeStraws. Throughout aging, effluent tubing directly emerging from LifeStraw outlets was routinely "pinched" to release build-up of air within LifeStraw (note: air was not present in influent tubing).

3.3 Challenge days

Challenge water refers to the water that was seeded with known concentrations of test microbes pumped through the LifeStraws on challenge days. Challenge water was dechlorinated tap water or dechlorinated tap-water amended with 1% pasteurized settled sewage to which target concentrations of test microbes were added for delivery to LifeStraws. The volume of water used to challenge LifeStraws varied from 25L to 30L depending on the challenge series and number of straws. Presence of chlorine in challenge water was tested using the Hach Total Chlorine kit prior to filling the challenge water tank.

Temperature was also measured in the challenge water tank before water use. Prior to pumping the challenge water to LifeStraws, each LifeStraw was backwashed, maintaining a pressure of 3 – 4 psi (0.2 – 0.275 bar) on the mouthpiece for 15 seconds; backwashing flow rate was not measured. After backwashing, the entire volume of the challenge water was pumped through typically 10 LifeStraws set up in parallel; the effluent water from each LifeStraw was collected in 3L containers.

As the challenge water was pumped through, an effluent sample was collected from each LifeStraw for chemical analyses. Total iodine (iodide + iodine) was tested using the Taylor Midget Comparator Test. If there was detectable iodine, presence of iodide was tested using the same test kit. Effluent water was also tested for presence of silver using the Hach Rapid Silver test kit. When the entire volume of the challenge water had been collected as effluent, the sample containers were immediately moved into the pathogen laboratory for microbial analysis. LifeStraws were backwashed following the challenge period, after which the aging procedure was resumed. Throughout the experiment, the flow rate for each LifeStraw was monitored and adjusted as necessary to maintain ~150 ml/min.

3.4 Chemical tests

The presence of two chemical disinfectants, silver and iodine, was monitored in the effluent water from the LifeStraws throughout the experiments. The presence of residual chlorine was also monitored in the aging and challenge water in order to insure no chlorine presence in tap water that was used to formulate aging and challenge water. On challenge days, 45ml samples of LifeStraw effluent waters were collected directly from drain tubes. The remainder of the effluent water was collected in 3L containers, each of which contained 100µl of a 2% sodium thiosulfate solution used to quench any remaining free iodine.

Quenching residual iodine was an important step when testing a water treatment device such as the LifeStraw. This is because residual iodine discharged from the LifeStraw could continue to act on microbes in test water, However, under real-use conditions the LifeStraw effluent water immediately enters the consumer's body and there is no further contact time between microbes and residual iodine disinfectant.

3.4.1 Iodine and iodide

The Taylor colorimetric midget test kit was used for the detection of iodine and iodide in effluent water from the LifeStraws. The Taylor kit indicates concentrations between zero, and two parts per million. The Taylor test kit is a two stage kit: the first stage tests for presence of total iodine, the second stage tests for presence of iodide. When there was no detectable presence of total iodine (iodine and iodide) in a sample, it was considered unnecessary to test for iodide.

3.4.2 Silver

Hach Rapid Silver test kit was used to measure presence and concentration of silver in the effluent water from the LifeStraws. The Hach test is a colorimetric test that detects concentrations from 5-50 parts per billion.(ppb) Concentrations in excess of 50 ppb were detected by making a known volumetric dilution of the sample in reagent water

3.4.3 Chlorine

The Hach Total Chlorine kit was used to measure the presence and concentration of residual chlorine in tap-water that was dechlorinated with granular activated carbon prior to preparing aging and challenge water to be passed through the LifeStraws. The Hach test kit is a colorimetric test that measures concentrations for two different ranges: 0-0.7 mg/L and 0-3.5mg/L.

3.5 Preparation of test indicator microbes

3.5.1 *E. faecalis*

E. faecalis, ATCC strain 29212, was purchased and received on 3/2/2007. The strain was streaked onto a plate of Bile Esculin Azide (BEA) agar; the plate was inverted, and incubated overnight at 37°C. The following day material from an isolated colony was selected from the incubated plate and inoculated into 25 ml of TSB. The culture was incubated overnight at 37°C with shaking. The following day, the broth culture was transferred into conical tubes and centrifuged at 14K for 5 minutes, the supernatant was removed and the pellet re-suspended in 25 ml of tryptic soy broth (TSB) with 20% glycerol. The final broth culture with glycerol was aliquoted into approximately 1 ml portions and stored at -80°C until needed to create spiking culture for experiments. To propagate a high titer of *E. faecalis* to use as spiking stock for challenge experiments, a broth culture method was used. An overnight culture was inoculated into 25 ml TSB two nights before the first challenge experiment and incubated at 37^{o}C with shaking. (Clesceri, 1992) On the third day a log-phase culture was prepared, incubated at 37^{o}C with shaking for 3 hours, then tittered using standard membrane filtration procedure and BEA agar.

3.5.2 MS2 coliphage and double agar layer (DAL) propagation and assay method

The EPA DAL method was used to prepare a stock of MS2 coliphage. Known MS2 and *E. coli* F_{amp} control strains were obtained within the Sobsey laboratory inventory. *E. coli* Famp host cells were infected with MS2 within agar medium-host cell lawns using dilutions at which discrete MS2 viral plaques developed. An isolated viral plaque was extracted from the agar medium and enriched in TSB containing *E. coli* F_{amp} host cells and and streptomycin

and ampicillin at a concentration of 15μg/ml; the broth was incubated with shaking at 37°C for 18 – 24 hours.

After incubation the infected broth culture material was subjected to extraction with a half volume of Freon and emulsified by shaking for 5 minutes to partially purify viral particles in stock broth culture. The mixture was then centrifuged at 2500x g for 20 minutes at 4°C. The top aqueous layer of supernatant containing viral particles was poured into 150mm Petri dishes and placed open under a biological hood for 30 minutes to allow any remaining Freon to evaporate. Freon-extracted viral particles were aliquoted as small volumes, stored at -80°C and thawed as needed prior to the challenge experiment. (U. S. EPA, 2001)

3.5.3 MS2 coliphage plaque assay by the single agar layer method

The single agar layer (SAL) method was used in the LFO7 and F series instead of the DAL method for time efficiency and the expectation of reliable results. *E. coli* Famp host cells were infected within the developing lawn of a single agar medium plus host cells to develop MS2 viral plaques. An isolated viral plaque was extracted from the agar medium and enriched in a broth composed of TSB, *E. coli* F_{amp} host cells, and streptomycin and ampicillin at a concentration of 15μg/ml; the broth was incubated with shaking at 37°C for 18 – 24 hours.

Freon extraction was used to partially purify viral particles in infected stock broth culture material as described above. Freon-extracted viral particles were aliquoted into small volumes, stored at -80°C and thawed as needed prior to challenge experiments. (U. S. EPA, 2001)

3.5.4 *E. coli* KO11

A known *E. coli* KO11 control strain was obtained from the Sobsey laboratory inventory. The strain was streaked onto Biorad Rapid *E. coli* 2 agar containing 40 μg/ml chloramphenicol, inverted, and incubated overnight at 37°C. The following day an isolated colony with the expected appearance was selected from the incubated plate and inoculated into 25 ml of TSB containing 40 μg/ml chloramphenicol. The culture was incubated overnight at 37°C with shaking. The following day, the broth culture was transferred into conical tubes and centrifuged at 14K for 5 minutes, the supernatant was removed and the pellet re-suspended in 25 ml of TSB with 20% glycerol. The final broth culture with glycerol was aliquoted into approximately 1 ml portions and stored at -80°C until needed for experimental use. To propagate a high titer of *E. coli* KO11 to use as spiking stock for challenge experiments a small amount of frozen stock was inoculated into 25 ml of TSB with chloramphenicol, and incubated at 37°C with shaking two nights before the first challenge experiment

3.5.5 *C. perfringens*

A known *C. perfringens* control strain was obtained within the Sobsey laboratory inventory. An isolated colony grown by streak plate on mCP agar, the following day an isolated colony was selected, cultured in 100 ml of mCP broth, and incubated overnight at 44°C in a BBL GasPak anaerobic chamber. The third day, the broth was transferred into two 50 ml conical tubes and centrifuged at 3000 rpm and 4°C for 10 minutes. The supernatant was removed and the remaining pellet was re-suspended in 25 ml of 7.5 pH, phosphate buffered saline (PBS). The sample was vortex mixed, then centrifuged again at 3000 rpm and 4°C for 10 minutes. The supernatant was removed again, and then the pellet was re-suspended in 2.5 ml PBS. The two concentrated sample pellets were combined and spread

plated on each of 20 modified Duncan-Strong sporulation agar plates. The plates were incubated for 48 hours at 44°C anaerobically.

At 48 hours, spore crops were harvested from plates by gently scraping spores from the agar surface. A 5 ml volume of PBS was used to rinse remaining spores from each plate (5 plates per 50 ml conical tube for a total of 4 conical tubes). Harvested spores were washed by centrifuging at 3000 rpm and 4°C for 10 minutes, the supernatant was removed and the remaining pellet was re-suspended in 25 ml PBS. The sample was vortex mixed, then centrifuged again at 3000 rpm and 4°C for 10 minutes. The spore suspensions were heat-treated at 70°C for 30 minutes to kill any remaining vegetative cells. Spores were then washed by centrifuging at 3000 rpm and 4°C for 10 minutes, the supernatant was removed and the remaining pellet was re-suspended in 25 ml PBS. The sample was vortex mixed, then centrifuged again at 3000 rpm and 4°C for 10 minutes. Supernatant was removed again and the pellet was re-suspended in 8 ml of PBS. Suspensions were combined for a total of 32 ml.

The spore concentration was 10^5 spores/ml at this stage, as determined by viable count using the spread plate method. A 10-fold dilution series was created by serially transferring 1 ml of spore suspension into 9 ml PBS. Dilutions 10^{-1} through 10^{-4} were spread plated in duplicate on tryptose sulfite cycloserine (TSC) agar. Plates were inverted then incubated anaerobically in BBL GasPak anaerobic chambers overnight at 44°C. The following day the plates were read for colony counts, the titer was determined, and the volume of log phase culture to spike into challenge water was calculated. Spore suspensions were divided into three 8-ml volumes and stored at 4°C until day of challenge experiments.

3.6 Preparation of influent water

Two separate 15-liter volumes of challenge water were prepared. One container received 15 liters of dechlorinated tap water; while the other received 15 liters of dechlorinated tap water with 1% pasteurized settled sewage. Indicator microorganisms were added (spiked) to each of the two 15-L volumes of challenge water to achieve the following final concentrations:

- *E. faecalis*: 10^6 CFU/100 ml (used in all four series)
- *E. coli* B: 10^6 CFU/100 ml (used for LF07 and F series)
- *E. coli* KO11: 10^6 CFU/100 ml (only used for NVO-YAO series)
- MS2 coliphage: 10^6 PFU/100 ml (used in all four series)
- C. perfringens: 10^5 CFU/100 ml (only used for NVO-YAO series)

Following the additions of test microbes, challenge water was mixed for 30 minutes using a stir bar and plate prior to pumping it through the LifeStraw system.

Enumeration of indicator microbes in influent challenge water

3.6.1 *E. coli* KO11 and *E. coli* B

A serial ten-fold dilution series through 10^{-5} was made by transferring 2 ml of influent water into 18 ml of PBS. Membrane filtration was done on 9-ml volumes of appropriate dilutions in duplicate on Bio-Rad Rapid *E. coli* 2 agar (for *E. coli* KO11 the medium also contained 40 μg/ml chloramphenicol). Plates were inverted then incubated at 37°C for 18-24 hours. Colonies were counted and concentrations were expressed as colony forming units (CFU)/ml.

3.6.2 *E. faecalis*

A serial ten-fold dilution series was made through 10^{-5} by transferring 2 ml of influent water into 18 ml of PBS. Membrane filtration was done on 9-ml volumes of appropriate dilutions in duplicate on BEA agar. Plates were inverted then incubated at 37°C for 18-24 hours. Colonies were counted and concentrations were expressed as CFU/ml

3.6.3 MS2 coliphage

A serial ten-fold dilution series was made through 10^{-5} by transferring 2 ml of influent water into 18 ml of TSB. The DAL or SAL plaque assay methods were used to enumerate MS2 in these dilutions as previously described above. Inoculum volumes per plate were 9 ml for the DAL and SAL methods. Plates were inverted then incubated at 37°C for 18 to 24 hrs. Plaques were counted and concentrations were expressed as plaque forming units (PFU)/ml (U. S. EPA, 2001)

3.6.4 *C. perfringens*

For the enumeration of *C. perfringens* in influent water, 250 ml of influent sample was taken and heat treated at 60°C for 30 minutes. Three volumes of effluent were membrane filtered in duplicate; (1) 1ml of sample in 9 ml PBS, (2) 9 ml of undiluted sample, and 90 ml of undiluted sample. The filters were then placed on TSC agar containing 40 µg/ml cycloserine. Serial ten-fold dilutions were made by transferring 1 ml of the effluent water into 9 ml of PBS. A 100 µl volume of dilutions 10^{-2} through 10^{-5} were spread plated in duplicate on TSC agar containing 40 µg/ml cycloserine. Plates were inverted and incubated anaerobically at 44°C for 18-24 hours. Colonies that appeared to be *C. perfringens* were counted and concentrations were expressed as CFU/ml.

3.7 Enumeration of indicator microbes in effluent water

3.7.1 *E. coli* KO11 and *E. coli* B

Three volumes of effluent were membrane filtered in duplicate; (1) 1ml of sample in 9 ml PBS, (2) 9 ml of undiluted sample, and (3) 90 ml of undiluted sample. Filters were placed on Bio-Rad Rapid *E. coli* 2 agar (for *E. coli* KO11 40 µg/ml chloramphenicol was added). Plates were inverted and incubated at 37°C for 18-24 hours. Distinctive E. coli colonies were counted and concentrations were expressed as CFU/ml.

3.7.2 *E. faecalis*

Three volumes of effluent were membrane filtered in duplicate; (1) 1ml of sample in 9 ml PBS, (2) 9 ml of undiluted sample, and 90 ml of undiluted sample. Filters were placed on BEA agar plates, and plates were inverted and incubated at 37°C for 18-24 hours. Distinctive *E. faecalis* colonies were counted and concentrations were expressed as CFU/ml.

3.7.3 *MS2 coliphage*

SAL or DAL assay methods for MS2 coliphage were performed using the following four sample volumes; (1) 2 ml of sample in 18 ml of TSB, 0.2 ml of sample in 19.8 ml of sample, (2) 0.02 ml of sample in 19.98 ml of TSB, (3) 0.002 ml of sample in 19.998 ml of TSB, and (4) 0.0002 ml of sample in 19.9998 ml of TSB. Plates were inverted and incubated at 37°C overnight. The following day, plaques on plates were enumerated and MS2 concentrations were expressed as plaque forming units /ml. (U. S. EPA, 2001)

3.7.4 *C. perfringens*

For the detection of *C. perfringens* spores in effluent water, 250 ml of effluent was taken from each LifeStraw and heat treated at 60°C for 30 minutes. Three volumes of effluent were membrane filtered in duplicate; (1) 1ml of sample in 9 ml PBS, (2) 9 ml of undiluted sample, and (3) 90 ml of undiluted sample. The filters were then placed on TSC agar containing 40 µg/ml cycloserine. A serial ten-fold dilution was made by transferring 1

ml of the effluent water into 9 ml of PBS. Volumes of 100 µl of dilutions 10^{-1} through 10^{-3} were spread plated in duplicate on TSC agar containing 40 µg/ml cycloserine. Plates were invert and incubated anaerobically at 44°C for 18-24 hours. Distinctive C. perfringens colonies were counted and concentrations were expressed as CFU/ml.

3.8 Data collection

Following the determination of LifeStraw effluent titers, comparisons were made between influent and effluent concentrations of test microbes to compute $\log_{10}$ microbe reductions. Data were compiled for each challenge experiment using Excel worksheets. Microorganism concentrations were calculated as described in US EPA Method 1601. For each microorganism, $\log_{10}$ reduction values at each challenge point were calculated by subtracting the $\log_{10}$ of the effluent concentrations from the $\log_{10}$ of the influent concentrations. When no bacteria CFUs or virus PFUs were detected in the total volume assayed, the lower detection limit of the assay had been reached. In this case, a $\log_{10}$ reduction value was calculated using 1 CFU or PFU in the total sample volume assayed. This has been indicated by the use of a less-than symbol for the $\log_{10}$ concentration and a greater-than-or-equal-to symbol ($\geq$) for the $\log_{10}$ reduction. Overall $\log_{10}$ reduction values for each LifeStraw (over the total 700 L water volume of ageing) were calculated using total number of CFUs or PFUs in all challenge experiments and total volumes assayed, compared to the average concentration of test microbes in the challenge waters.

3.9 Data management

When no bacteria CFUs or virus PFUs are detected in the total volume assayed, the lower detection limit of the assay has been reached. In this case, a $\log_{10}$ reduction value is

calculated using 1 CFU or PFU in the total sample volume assayed. This is indicated by the use of a less-than symbol for the $\log_{10}$ concentration and a greater-than symbol (>) for the $\log_{10}$ reduction. Lifetime $\log_{10}$ reduction values for each LifeStraw represent lower bound estimates of log-reduction because they are calculated using the minimum threshold values.

3.10 LifeStraw series-specific experimental objectives, test conditions and methods

3.10.1 NVOYAO series objectives

1. Determine the efficacy of LifeStraws LF64 NVO and LF64 YAO to reduce the concentrations of the following indicator microbes in challenge water:
    a. *Enterococcus faecalis*
    b. *Escherichia coli* KO11
    c. *Clostridium perfringens* spores
    d. MS2 coliphage
2. Determine the residual concentrations of iodine, iodide, and silver released into effluent water

3.10.1.1 Additions and variations to general experimental design

The first challenge run was not preceded by an initial aging run.

3.10.2 C series objectives, test conditions and methods

1. Age five C series LifeStraws with ~700 L dechlorinated tap water
2. Challenge LifeStraws with indicator microorganisms representing two major microbial classes:
    a. Bacteria: *Enterococcus faecalis*
    b. Virus: MS2 coliphage

3. Consistently measure release of iodine and silver from LifeStraws into effluent water over time

3.10.2.1 Additions and variations to general experimental design

(1) Each LifeStraw was aged with ~18 L of dechlorinated tap water prior to the first challenge. (2) Measurement of total iodine, chlorine, temperature and silver in test waters did not begin until the second challenge (6/20/07).

3.10.3 L series objectives, test conditions and methods

1. Age five different LF07 series LifeStraws with ~700 L dechlorinated tap water
2. Age five different LF07 series LifeStraws with ~700 L dechlorinated tap water supplemented with 1% pasteurized settled sewage
3. Challenge LifeStraws with microorganisms representing two major classes:
    a. Bacteria:
        i. *Escherichia coli* B
        ii. *Enterococcus faecalis*
    b. Virus: MS2 coliphage

3.10.3.1 Additions and variations to general experimental design

(1) Approximately 150 ml of the initial effluent water from each LifeStraw were discarded to ensure that the collected effluent was solely microbe-seeded challenge water. (2) Backwashing was routinely performed after every 45 L of test water was passed through LifeStraws or when flow rate dropped below 125 ml/min. (4) On aging days, LifeStraws received anywhere between 8 and 67.5 L of ageing water.

3.10.4 F series objectives, test conditions and methods

1. Age five LF64 series and five LF07-008B LifeStraws with 700 L dechlorinated tap water with 1% pasteurized settled sewage
2. Challenge LifeStraws with indicator microorganisms representing two classes
    a. Bacteria:
        i. *E. coli* B
        ii. *E. faecalis*
    b. Virus:
        i. MS2 coliphage

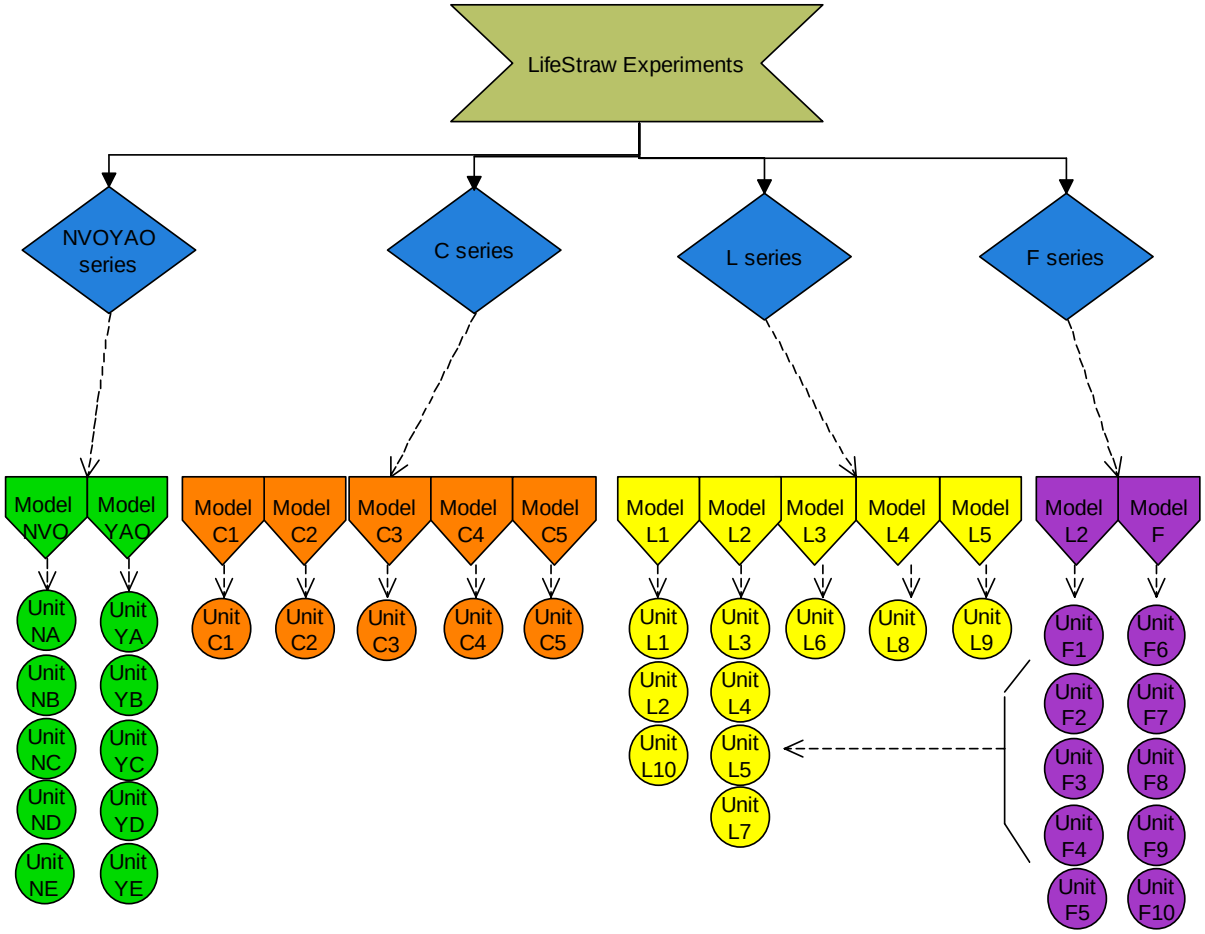
RESULTS

4.1 Preamble

Fifteen LifeStraw models were tested over four experimental series. Throughout the experiments there were measurable results for three test microbes (*E. faecalis*, *E. coli*, MS2) using two water types for aging and challenging. For some of the LifeStraw models there were a number of replicates tested, however, the majority of models were represented by only one test unit. Figure 10 illustrates the testing dichotomy for the LifeStraw experiments.

Results from the experiments are presented principally by model type. Results are presented for each model by first presenting overall lifetime reductions, followed by evaluation of trends in reduction over aging volume, and chemical analysis of effluent water. Following the results by model is a section that addresses differences in reduction values by water type used for aging, a section that compares reduction of test microbes across model types, and a section that compares cumulative reduction capability of the three model types with the most robust results.

Figure 10: Flow chart for the LifeStraw experiments



4.2 Data management and statistics

The performance indicator value used throughout the LifeStraw experiments was the $\log_{10}$ reduction capability of specified test microbes. The $\log_{10}$ reduction value (LRV) is based on the difference in $\log_{10}$ microbe concentrations of the influent/seeded test water and the concentrations in the corresponding effluent water. For example, if the $\log_{10}$ concentration of *E. faecalis* was $1x10^6$ in the influent water and $1x10^2$ in the effluent water, the representative LRV would equal 4 $\log_{10}$. However, if the $\log_{10}$ concentration of *E. faecalis* was low in the influent assay methods would not be capable of detecting any growth of the test microbe. When no bacteria CFUs or virus PFUs are detected in the total volume

of a LifeStraw challenge water filtrate assayed, the lower detection limit of the assay has been reached. In this case, a $\log_{10}$ reduction value is calculated using 1 CFU or PFU in the total sample volume of the challenge water LifeStraw filtrate assayed. This is indicated by the use of a less-than symbol for the $\log_{10}$ concentration and a greater-than symbol (>) for the $\log_{10}$ reduction. Overall $\log_{10}$ reduction values for each LifeStraw (over total 700 L water volume of aging) represent lower bound estimates of log-reduction values because they are calculated using the minimum threshold values for $\log_{10}$ microbe concentrations, expressed as less-than values. The detection limits of the microbe assays change for each challenge day. This is because the detection limit is defined by both the volumes of LifeStraw challenge water influents and their corresponding filtered effluents assayed and by the influent microbe concentrations in the seeded challenge water influents. Challenge days that had lower than intended influent microbe concentrations that did not allow detection of at least a 6 $\log_{10}$ microbe reduction by the LifeStraw inadequately reflect on its ability to meet the 6 $\log_{10}$ reduction target set by US EPA and NSF-International for performance certification. When greater than $\log_{10}$ reductions values were lower than the performance target reductions, they are indicated by a dagger (†). While greater-than symbols indicate that there is uncertainty in extent above the detection limit that the real LRV lies, the dagger symbol indicates the mean lifetime LRV could possibly be below the target reduction due to the detection limit of the assay, ***not*** the reduction ability of the LifeStraw. Reduction of *E. coli* and *E. faecalis* by test LifeStraws were often higher than the detection limit of the assay.

Log_{10} microbe reduction data were entered into a Microsoft Excel spreadsheet and copied into GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). All data were verified to ensure consistency and

accuracy of data input. Log reduction values (LRVs) were stratified by microbe, water type, challenge volume, and replicate. Statistical methods used include linear regression (with 95% confidence intervals), ANOVA, and standard deviation. Linear regression was used to describe trends over the volume of aging water applied to the LifeStraw. Statistical ANOVA were used to describe patterns of $\log_{10}$ reduction among model types, microbes, and water types. Means comparison was also done using Tukeys HSD (Honestly Significantly Different) method. Assumptions made in comparing LRV data in statistical testing were that data were presented a Gaussian distribution and groups had equal variances (these assumptions were specified by GraphPad). All tests were compared using a significance level (P-value) threshold of 0.05.

Of the 15 LifeStraw models that were tested, three models (L1, L2, F) provided an adequate sample size (n) for statistical analysis. Thorough analysis of these three models was of particular importance to the manufacturer, VF, because they were the models expected to achieve the best overall performance as candidates to market. Results from the remaining 12 models are presented qualitatively showing the arithmetic mean of the LRV's sequentially throughout the aging volume. It should be noted that for all LifeStraw models tested the sample size was too small for explicit outcome predictions to be made. Ideally, a sample size (n) of more than 30 would be appropriate for robust statistical analysis.(Ahn, 2006) Because of manufacturer priorities and design LifeStraw experiments, it was not possible to test enough LifeStraws to get an *n* of 30 for any of the models. Although no published research on the microbial reduction capacity of the LifeStraw exists, similar work has been done on iodinated resin purifiers. This work provides insight into the effectiveness and abilities of the LifeStraw, as well as supporting significant performance trends that we

found in our research (Clasen & Menon, 2007; Clasen, Nadakatti, & Menon, 2006; Schlosser et al., 2001).

4.3 Test water characteristics

Throughout the challenge experiments, two types of test water were used for aging and challenging. In most cases, test LifeStraws were only aged with de-chlorinated tap water (DTW). .In order to more realistically simulate influent water used by consumers of the product, some L series LifeStraws and all the F series LifeStraws were aged using de-chlorinated tap water with 1% pasteurized settled sewage (DTW+SS). The physiochemical composition of the aging waters used was characterized by analyzing the pH, turbidity, total organic carbon (TOC), total dissolved solids (TDS), temperature at use, and the presence of chlorine. Tables 3-5 show the results of the analysis. Water quality guidelines for aging water were taken from the USEPA *Guidelines for Testing Microbiological Water Purifiers* (EPA, 1987)

Water type DTW and DTW+SS had similar pH, temperatures, and concentrations of chlorine. Overall pH for the two water types was ~8.3, while the temperature of the aging water at the time of use ranged from 24°C to 27°C. No chlorine was detected in either aging waters. As expected, DTW+SS water type had a higher turbidity (DTW+SS: 0.46 NTU and DTW: 1.6 NTU) than de-chlorinated tap water alone. Aging water with settled sewage added also had higher TDS and TOC; mean values for TDS were 720 mg/L in DTW+SS and 443 mg/L in DTW, TOC means were 0.44 mg of C/L for DTW+SS and 0.206 mg of C/L for DTW.

Table 3: Aging water measurements: pH and turbidity (NTU)

	pH	Turbidity (NTU)

DTW A	8.10	0.238
DTW B	8.48	0.096
DTW C	8.40	0.130
Average DTW	**8.33**	**0.155**
DTW + SS A	8.30	0.455
DTW + SS B	8.29	0.459
DTW + SS C	8.24	0.462
Average DTW + SS	**8.28**	**0.459**

Table 4: Aging water measurements

	TDS (mg/L)	TOC (mg of C/L)
DTW A	290	0.258
DTW B	540	0.226
DTW C	500	0.134
Average DTW	**443**	**0.206**
DTW + SS A	320	0.516
DTW + SS B	880	0.386
DTW + SS C	960	0.408
Average DTW + SS	**720**	**0.437**

Table 5: Temperature and chlorine concentrations of aging water prior to challenge experiments

	Vol. at Challenge (L)		Temperature	Chlorine
Date	**L2 - L9**	**L1, L10**	**(^{o}C)**	**(mg/L)**
6.29	10	ND	ND	BMDL
7.03	100	ND	24	BMDL
7.05	200	ND	24	BMDL
7.10	300	ND	24	BMDL
7.12	400	10	26	BMDL
7.17	500	100	25	BMDL
7.19	ND	200	25	BMDL
7.26	700	300	25	BMDL
7.31	ND	400	ND	BMDL
8.06	ND	500	26	BMDL
8.14	ND	700	27	BMDL

BMDL: below method detection limit (chlorine: 0.1 mg/L)
ND: no data

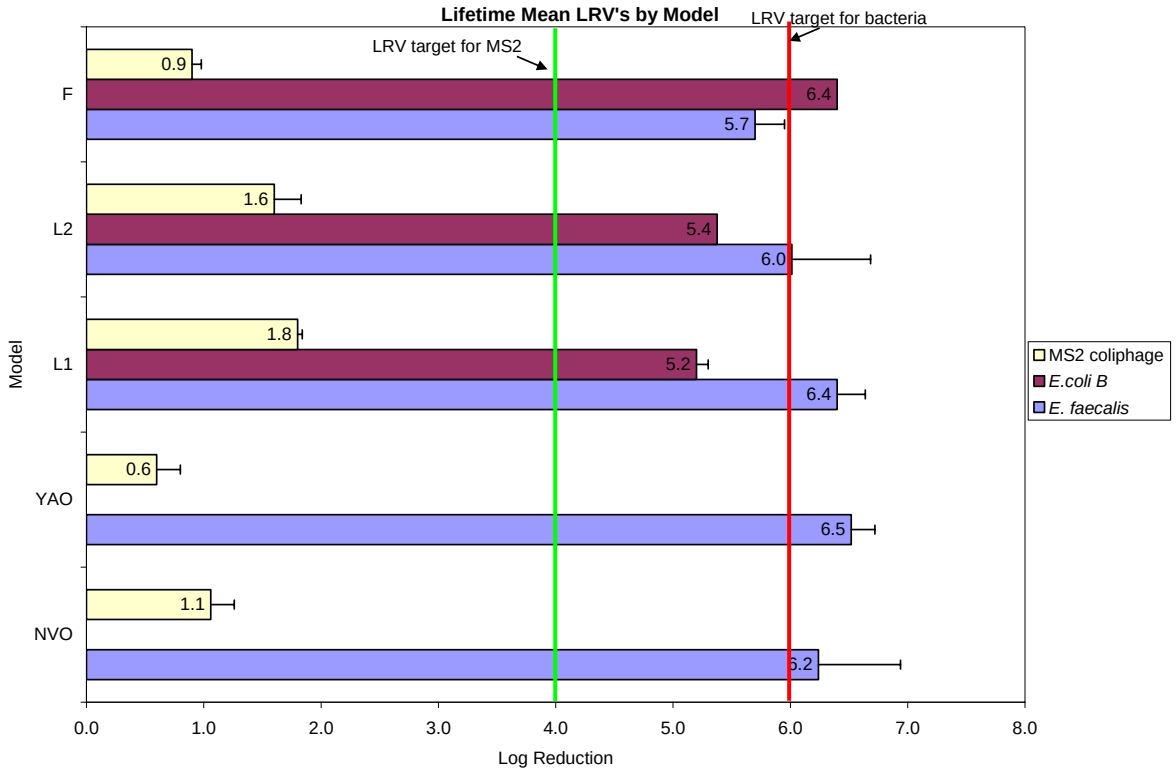
4.4 Lifetime LRV's by LifeStraw model and test microbe

Lifetime LRV represents the mean ability of a LifeStraw to reduce concentrations of a test microbe from sequentially applied challenge water seeded with test microbes over a total 700L of aging water. Lifetime LRV's were calculated for each unit by taking the arithmetic mean of LRV's for each challenge point. In order to calculate the lifetime LRV for each model, the arithmetic mean of the lifetime LRV for each replicate unit was taken. The lifetime LRV was the primary performance indicator with which LifeStraw models were compared. Figure 11 presents lifetime LRVs of five LifeStraw model types for the three test microbes, *E. coli*, *E. faecalis* and coliphage MS2. Two vertical lines have been added to the chart to indicate the log reduction targets for the three microbes, the green line is the target for MS2, and the red line is the target for both *E. coli* and *E. faecalis*. Both *E. coli* and *E. faecalis* have mean lifetime LRV's that include minimum threshold values (greater-than values). When the microbial reduction exceeds the detection limit of the assay, all that can be said is that the LRV is greater than the maximum detectable LRV of the assay method. The minimum threshold value then represents the lowest LRV possible and allows the LRV to be treated as a real number. The implication of averaging minimum threshold LRVs is that the mean lifetime LRV can represent reduction that is lower than the real ability of the LifeStraw.

None of the five LifeStraw model types achieved the target 4.0 LRV for MS2. Model L1 had the highest LRV for MS2 (1.8 $\log_{10}$), model YAO had the lowest LRV for MS2 (0.75 $\log_{10}$). Four of the five model types met the target LRV for *E. faecalis*; model type F did not meet the target LRV but came close, with a 5.7 $\log_{10}$ reduction. Model F was the only one of the three models successfully tested for *E. coli* reduction to achieve higher than the 6 $\log_{10}$ reduction target. While Models L2 and L1 did not meet the 6 $\log_{10}$ reduction target, their

performance came close to it, with LRVs of 5.4 and 5.2, respectively. *E. coli* was not used as a test indicator bacterium for the NVO and YAO model LifeStraws. The decision not to use the *E. coli* was made in order to keep the experimental design manageable because the NVO and YAO series was the first of its kind.

Figure 11: Mean lifetime LRV's for each LifeStraw model tested



4.5 Model L1 results

The sample size for model L1 included three model replicates. Variables included: 1)two water types- dechlorinated tap water and dechlorinated tap water with 1% pasteurized settled sewage, 2)three microbes (*E. faecalis, E. coli*, MS2), and 3)seven observations at 10L, 100L-500L, and 700L. Replicate units L1 and L2 were aged and challenged with DTW and L10 was aged and challenged with DTW+SS. Regression charts and results tables for model L units can be seen in Table 6 and Figures 12-14.

Table 6: Test microbe LRVs and chemical concentrations for model L1 LifeStraws at each challenge interval for aging water

E. faecalis	**10**	**100**	**200**	**300**	**400**	**500**	**700**	Geometric Mean
L1	>7.0	>6.9	6.6	>5.9†	4.8	ND	ND	>6.2†
L2	>6.8	>6.8	>7.0	6.5	6.9	>6.9	>5.9†	>6.7†
L10	>7.0	6.5	6.3	>5.8†	6.2	ND	6.2	>6.3†
E. coli B								**6.4†**
L1	>4.2†	>4.8†	>6.0	>5.5†	>5.8†	>5.9†	>6.2	>5.5†
L2	ND	ND	>2.0†	6.0	>4.2†	>4.8†	>5.5†	>4.6†
L10	>4.3†	5.5	5.4	5.1	>5.6†	>6.2	>6.2	>5.5†
MS2								**5.2†**
L1	2.8	2.3	2.3	1.3	1.1	1.6	1.5	1.8
L2	2.4	1.7	1.4	1.6	2.6	1.9	1.0	1.8
L10	2.5	1.9	2.2	1.4	1.3	1.4	2.5	1.9
Iodine (mg/L)								**1.8**
L1	BMDL	BMDL	BMDL	BMDL	BMDL	BMDL	BMDL	
L2	ND	BMDL	BMDL	BMDL	BMDL	BMDL	BMDL	
L10	BMDL	BMDL	BMDL	BMDL	BMDL	BMDL	BMDL	
Silver (ppb)								
L1	18	20	35	25	8	BMDL	BMDL	21
L2	8	150	80	150	18	6	6	60
L10	18	40	10	6	5	BMDL	BMDL	16
ND: no data † is, or includes, a greater-than value lower than the 6 $\log_{10}$ target LRV BMDL: below method detection limit (Silver: 5 ppb; Iodine 0.1 mg/l)								**32.2**

E. faecalis experienced the highest overall mean reduction (6.4 $\log_{10}$) of the three test microbes. Reductions of *E. coli* were 5.2 $\log_{10}$ and reduction of MS2 coliphage was 1.8 $\log_{10}$. Both the *E. coli* and the *E. faecalis* LRV's included greater-than values that were lower than the target 6 $\log_{10}$ reduction. The majority of the data points for *E. faecalis* and *E. coli* LRVs were greater than values because the bacteria levels in seeded challenge water were lower than intended and because no bacteria were detectable in the assayed volume of the LifeStraw effluent water. Hence, many LRVs are detection limits and real LRVs are likely higher than these values. Because these detection limit LRVs are also reflected in the overall lifetime mean LRV, it is expected that the overall lifetime mean LRV is likely to be higher than shown.

LifeStraw effluent water samples analyzed for presence of iodine/iodide were found to be below the minimum detection limit (BMDL) at all challenge intervals over straw use. Silver was detected in many of the LifeStraw effluent water samples from the various challenge intervals. Silver concentrations for model type L1 ranged from 5 to 150 ppb, with an overall mean of 32 ppb. In each of the three model replicates, silver concentrations that had been as high as 35 to 150 ppb declined appreciably after the 300L challenge interval to 6 ppb or less.

4.5.1 Trends in LifeStraw microbial reductions over water aging volume

As shown in Figure 12, $\log_{10}$ reductions of *E. faecalis* were not correlated with volume filtered over the 700L aging water lifetime. By linear regression analysis using volume filtered as the independent variable, the R^2 value was 0.3 for pooled data. There was also little evidence of correlation of MS2 $\log_{10}$ reductions with aging water volume over time, and the 95% confidence interval was low ($R^2 = 0.5$). However, $\log_{10}$ reductions of *E. coli* significantly increase with aging water volume, and the confidence interval was low ($R^2 = 0.7$). Nearly all *E. coli* $\log_{10}$ reductions were greater-than (>) values and therefore censored estimates of the real $\log_{10}$ reduction performance of these LifeStraws. As efforts were being made to increase *E. coli* concentrations in seeded challenge water over the aging intervals, these increasing LRVs could be an artifact cause by those changes in experimental conditions.

Figure 12: Regression of *E. faecalis* LRV's over volume aged for model L1

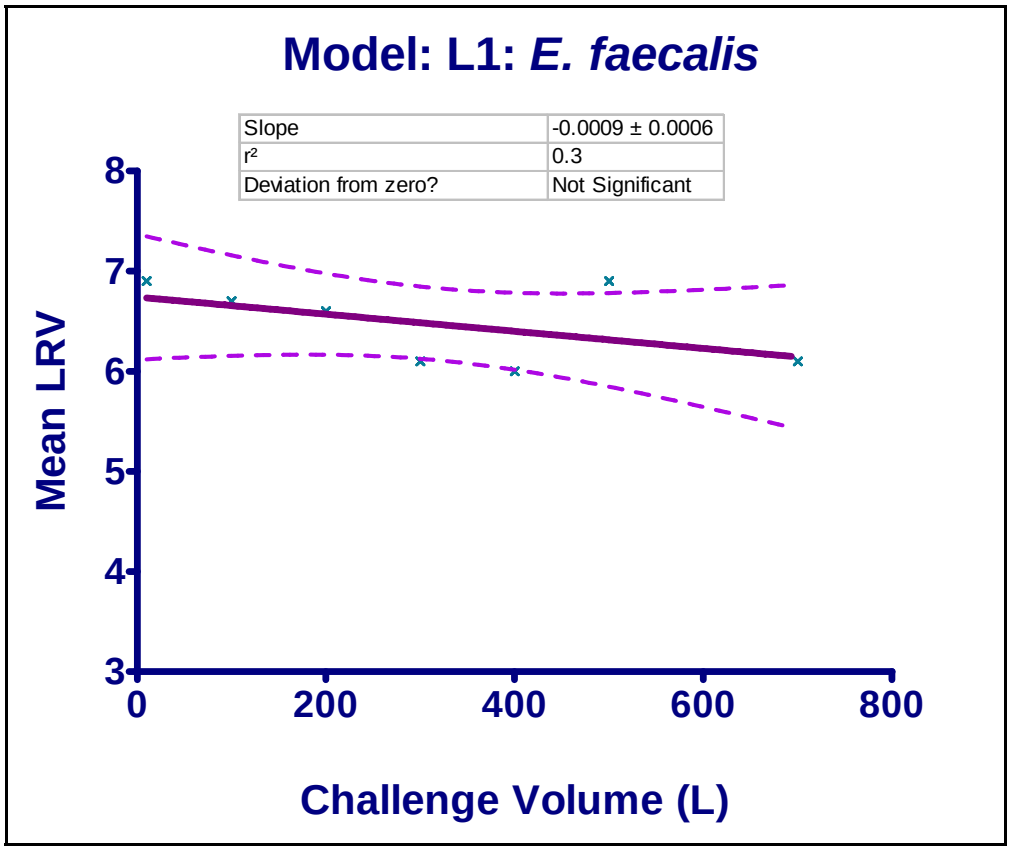


Figure 13: Regression of *E. coli* LRV's over volume aged for model L1

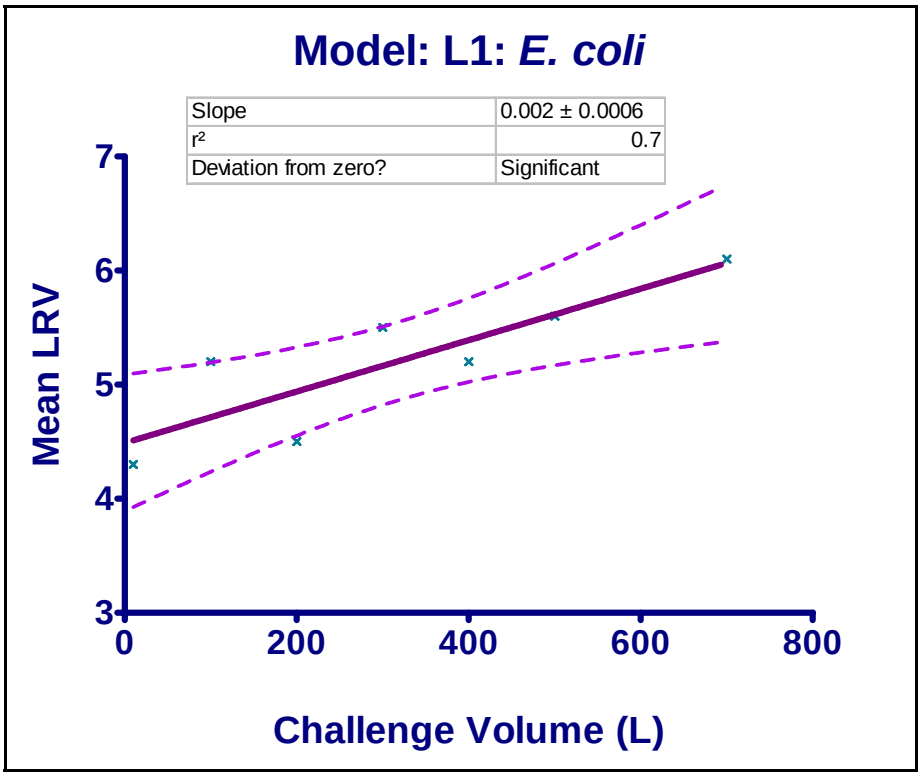
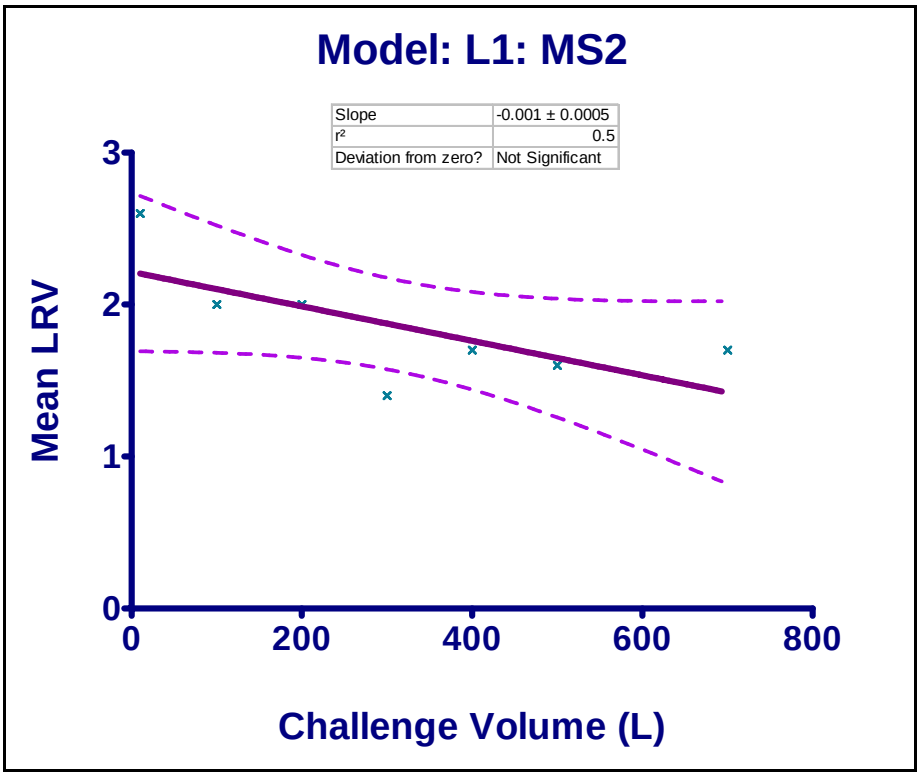


Figure 14: Regression of MS2 LRV's over volume aged for model L1



4.6 Model L2 results

The sample size for model L2 included nine model replicates. Replicate units were challenged in two series; units L3-L5 and L7 were tested in the L series and aged with de-chlorinated tap water only, units F6 – F10 were tested in the F series and aged with de-chlorinated tap water with 1% settled sewage. All nine model replicates were labeled by the manufacturer as the LF07-008B model type. Variables included two water types (DTW and DTW+SS), three microbes (*E. faecalis, E. coli*, MS2), and seven challenge water volume intervals after the following aging water volume: 10L, 100-500L in 100 liter increments, and 700L. Regression charts and results tables for model F units can be seen Table 7 and Figures 15-17.

Table 7: Test microbe LRVs and chemical concentrations for model L2 LifeStraws at each challenge interval for aging water

E. faecalis	**10**	**100**	**200**	**300**	**400**	**500**	**700**	**Mean**
L3 (dtw)	>6.8	>6.8	>7.0	6.5	>7.0	6.8	>5.9†	>6.7†
L4 (dtw)	>6.8	>6.8	>7.0	>6.5	>7.0	>6.9	>5.9†	>6.7†
L5 (dtw)	>6.8	>6.8	6.9	>6.5	>7.0	>6.9	5.9	>6.7
L7 (dtw)	6.6	>6.	>6.8	‡	‡	‡	‡	>6.
F6 (ss)	>6.9	>6.5	6.4	6.6	4.5	3.3	ND	>5.7
F7 (ss)	>6.9	>6.5	5.9	6.6	5.4	3.3	2.8	>5.3
F8 (ss)	>6.9	>6.5	6.4	6.6	6.4	3.3	2.8	>5.6
F9 (ss)	>6.9	>6.5	6.4	6.6	5.7	3.3	2.8	>5.5
F10 (ss)	>6.9	>6.5	6.4	6.6	6.1	3.3	0.6	>5.2
E. coli B								**6.0†**
L3 (dtw)	ND	ND	>2.0†	>6.1	>4.2†	>4.8†	5.4	>4.
L4 (dtw)	ND	ND	>2.0†	5.4	>4.2†	>4.8†	>5.5†	>4.
L5 (dtw)	ND	ND	>2.0†	>6.1	>4.2†	>4.8†	5.9	>4.
L7 (dtw)	ND	ND	>2.8†	‡	‡	‡	‡	>2.
F6 (ss)	>6.6	>6.0	>6.1	>6.2	>6.7	>6.6	>6.5	>6
F7 (ss)	>6.6	>6.0	>6.1	>6.2	>6.7	>6.6	>6.5	>6
F8 (ss)	>6.6	>6.0	>6.1	>6.2	>6.7	>6.6	>6.5	>6
F9 (ss)	>6.6	>6.0	>6.1	>6.2	>6.7	>6.6	>6.5	>6
F10 (ss)	>6.6	>6.0	>6.1	>6.2	>6.7	>6.6	>6.5	>6
MS2								**>5.4†**
L3 (dtw)	2.2	1.7	1.7	1.8	2.7	1.7	1.1	1.
L4 (dtw)	2.2	1.7	1.8	1.5	2.8	1.9	1.1	1.
L5 (dtw)	2.1	1.6	1.2	1.9	2.5	1.8	1.0	1.
L7 (dtw)	2.0	1.6	2.0	‡	‡	‡	‡	1.
F6 (ss)	2.9	1.8	1.0	1.7	1.4	2.0	1.1	1.
F7 (ss)	2.6	1.8	0.8	1.9	1.5	1.8	1.1	1.
F8 (ss)	2.6	1.8	0.8	1.8	2.1	1.8	1.1	1.
F9 (ss)	2.8	1.7	1.0	1.8	1.4	1.8	1.1	1.
F10 (ss)	2.6	2.0	1.2	1.8	1.5	1.8	1.1	1.
Iodine (mg/L)								**1.7**
L3 (dtw)	ND	BMDL	BMDL	BMDL	BMDL	BMDL	BMDL	
L4 (dtw)	ND	BMDL	BMDL	BMDL	BMDL	BMDL	BMDL	
L5 (dtw)	ND	BMDL	BMDL	BMDL	BMDL	BMDL	BMDL	
L7 (dtw)	ND	BMDL	BMDL	‡	‡	‡	‡	
F6 (ss)	BMDL	BMDL	BMDL	BMDL	BMDL	0.6	BMDL	
F7 (ss)	BMDL	BMDL	BMDL	BMDL	BMDL	0.4	BMDL	
F8 (ss)	BMDL	BMDL	BMDL	BMDL	BMDL	0.6	BMDL	
F9 (ss)	BMDL	BMDL	BMDL	BMDL	BMDL	0.2	0.4	
F10 (ss)	BMDL	BMDL	BMDL	BMDL	BMDL	0.4	0.2	
Silver (ppb)								
L3 (dtw)	15	15	150	200	12	6	5	77
L4 (dtw)	7	100	200	180	8	BMDL	BMDL	99
L5 (dtw)	10	15	100	200	20	BMDL	BMDL	96
L7 (dtw)	22	15	80	‡	‡	‡	‡	84
F6 (ss)	6		3	5	2	1	2	3

F7 (ss)	5	2		3	9	0	7	4
F8 (ss)	5	2	1	4	4	1	7	3
F9 (ss)	5	2	1	3	3	0	15	4
F10 (ss)	5	2	2	3	5	1	7	4
ND: no data	BMDL: below method detection limit			‡ LifeStraws removed from study due to leaking † is, or includes, a greater-than value lower than target LRV SS: 1% past. settled sewage DTW: de-chlorinated tap water				**42**

The overall lifetime arithmetic mean LRV's for model L2 were very similar to those of model L1. Of the three microbes tested, overall reductions were highest for *E. faecalis* at 6.0 $\log_{10}$, followed by *E. coli* at 5.4 $\log_{10}$ and lowest for MS2 coliphage at 1.6 $\log_{10}$. Mean lifetime model LRV's are calculated using LRV's from all nine replicates; by pooling all replicates the assumption is made that water type used for aging does not significantly impact reduction ability. This assumption is supported by results presented in a subsequent section that specifically addresses the influence of water type on microbial reduction.

For the majority of LifeStraw effluents, concentrations of iodine and iodide were below the minimum detection limit (BMDL = 0.1 mg/L). Iodine was not detected in effluents of the L models tested and was detected in the F modules tested only in challenge water effluents after again water intervals of 500 and 700 liters, which is towards the end of straw use life. The maximum detected iodine level was 0.6 mg/L, and all of it was in the form of iodide.

Silver concentrations in LifeStraw effluents were considerably higher in model replicates from the L series than in model replicates from the F series. The lifetime arithmetic mean silver concentration for all nine models was 42 ppb; however when the lifetime silver concentrations are divided by challenge series, the L series model is ~25 times higher than the F series units. However, the mean concentration for the L series model replicates was 88 ppb while the mean concentration for the F series replicates was 3.6 ppb.

Silver concentrations in effluents of some LifeStraws sometimes exceeded the WHO guideline value of 100 ppb. However, the mean concentrations of silver in LifeStraw effluents over the lifetime use volume of 700 L did not exceed this guideline concentration.

4.6.1 Trends in LifeStraw microbial reductions over aging water volume

Log_{10} reductions of *E. faecalis* were strongly inversely correlated with water volume filtered over the 700L aging lifetime. Linear regression using volume filtered as the independent variable yielded evidence of an association ($p < 0.05$) for data that pooled both challenge waters. The 95% confidence band for *E. faecalis* reductions was narrow ($R^2 = 0.9$). Log_{10} reductions of *E. coli* did not show a significant change in magnitude of LRV with aging water volume, confidence bands were also wide ($R^2 = 0.2$). Similarly, there was little evidence of change in magnitude of MS2 log_{10} reduction with increasing aging water volume, precision was low ($R^2 = 0.3$).

Figure 15: Regression of *E. faecalis* LRV's over volume aged for model L2

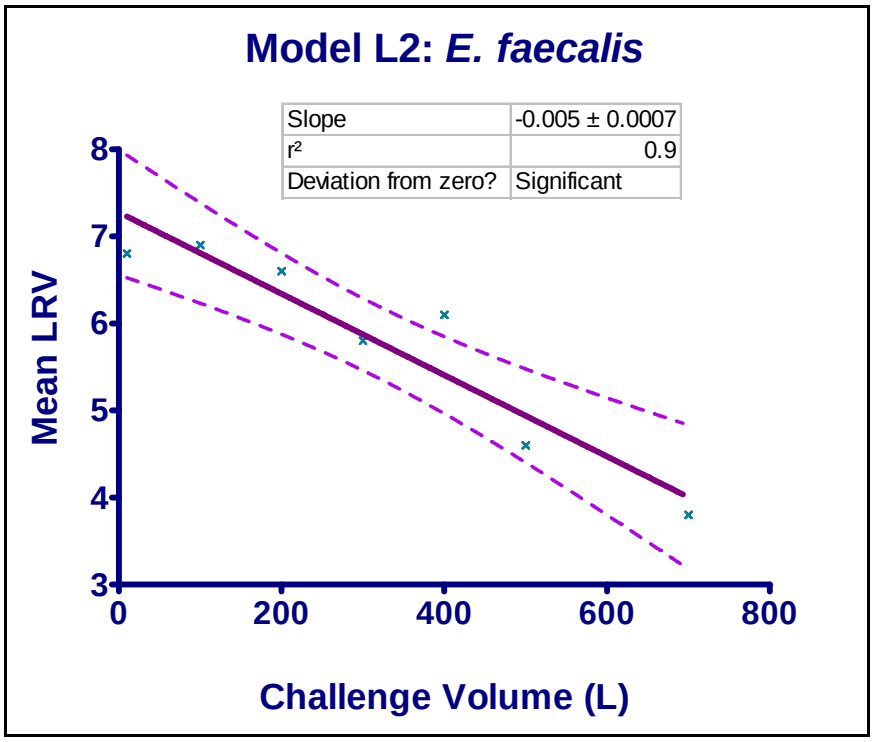


Figure 16: Regression of *E. coli* LRV's over volume aged for model L2

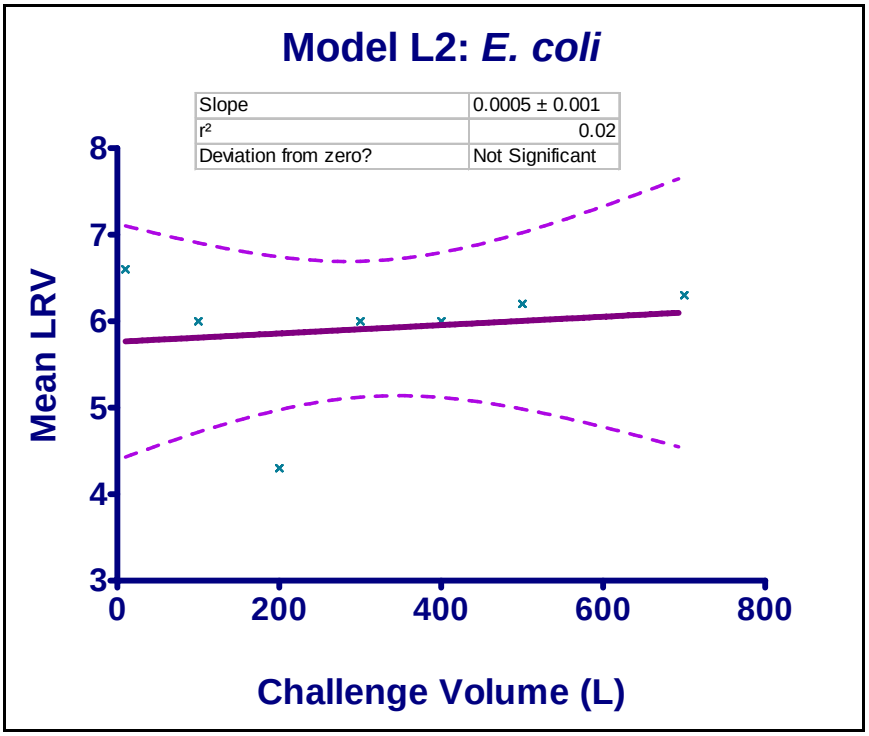
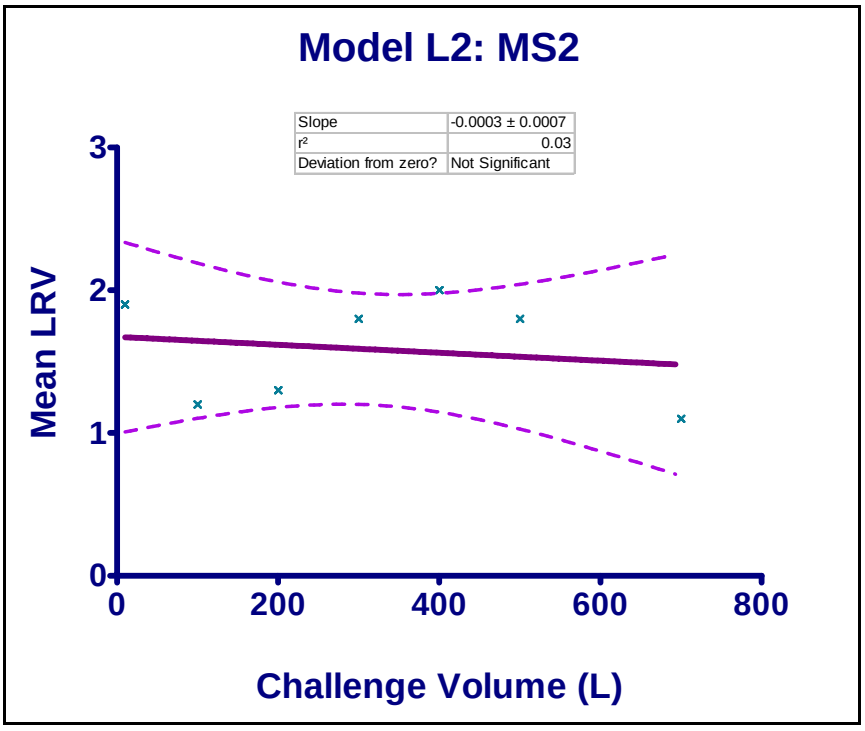


Figure 17: Regression of *MS2* LRV's over volume aged for model L2



4.7 Models L3 – L5 results

The sample size for LifeStraw models L3-L5 included only one replicate per model type. Models L3-L5 were aged and challenged with DTW+SS. Experimental variables included three test microbes (*E. faecalis, E. coli*, MS2), seven challenge water intervals at age water volumes of 10L, 100-500L in 100-liter increments, and 700L. The results of these experiments can be seen Table 8.

Although model L3, L4, and L5 share similarities in design with the other LFO7 models, each of the three LifeStraws differ by one physical variable (see Table 2). Similar to the circumstances for the C model LifeStraws, models L3-L5 do not provide a large enough data set to perform statistical analysis as done for other Lifestraw series for which there are more replicates. Nevertheless, it is possible to examine status and trends in the data for these straws and make performance comparisons among them. For *E. faecalis*, the overall mean LRVs ranged from 6.6 $\log_{10}$ to 6.8 $\log_{10}$ with standard deviations ranging from 0.1 $\log_{10}$ to 0.4 $\log_{10}$. The relatively low standard deviation relative to the mean reflects the large proportion of data that were "greater than" values. These "greater than" values were based on LifeStraw challenge water effluent concentrations that were below the detection limits of the *E. faecalis* assays and therefore, were expressed as "less than" values.

For *E. coli*, all of the LRV's exceeded the detection limits of the microbe assays and therefore, are calculated as "greater than" values for the same reasons as applied to *E. faecalis*. *E. coli* reductions ranged from >2.8 to >6.6 $\log_{10}$ in all challenges for which there were data (standard deviation 0.14 $\log_{10}$). However, a considerable proportion of the dataset was missing due to filter clogging and methodological problems in microbe assays. LRV's for MS2 were similar to those found in LifeStraw models L1 and L2. Mean lifetime LRV's

ranged from 2.2 $\log_{10}$ to 1.6 $\log_{10}$ with standard deviations ranging from 0.3 $\log_{10}$ to 0.4 $\log_{10}$.

All LifeStraw effluent samples had iodine/iodide concentrations below the minimum detection limit (BMDL of 0.1 mg/l). There were detectable concentrations of silver in all but one of the samples analyzed. The maximum detected silver concentration in a LifeStraw effluent water sample was 150 ppb, the while average effluent concentration for a LifeStraw model ranged from 105 ppb to 67 ppb. LifeStraw effluent silver concentrations from three consecutive challenges after 200L, 300L, and 400L, of aging water were approximately ten times higher than those for the other four challenges after 10, 400, 500 and 700L of aging water.

Table 8: Test microbe LRVs and chemical concentrations for model s L3-L5 LifeStraws at each challenge interval for aging water

E. faecalis	**10**	**100**	**200**	**300**	**400**	**500**	**700**	Mean LRV
L3	6.6	>6.8	>6.8	‡	‡	‡	‡	>6.8
L4	>6.7	>6.8	>6.8	>6.6	>7.0	6.9	>5.8	>6.7
L5	>6.7	6.7	>6.8	>6.6	>7.0	6.5	>5.8	>6.6
E. coli B								
L3	ND	ND	>2.8	‡	‡	‡	‡	
L4	ND	ND	>2.8	>6.1	>4.3	>5.6	>5.7	>4.9
L5	ND	ND	>2.8	>6.1	>4.3	>5.6	>5.7	>4.9
MS2								
L3	2.1	1.8	2.6	‡	‡	‡	‡	2.2
L4	2.0	1.6	1.5	1.6	2.0	1.6	1.1	1.6
L5	2.0	1.6	1.6	1.7	2.0	1.6	1.1	1.7
Iodine (mg/L)								
L3	ND	BMDL	BMDL	‡	‡	‡	‡	
L4	ND	BMDL	BMDL	BMDL	BMDL	BMDL	BMDL	
L5	ND	BMDL	BMDL	BMDL	BMDL	BMDL	BMDL	
Silver (ppb)								
L3	15	150	150	‡	‡	‡	‡	105.0
L4	20	80	100	150	5	BMDL	6	71.0
L5	15	150	80	150	5	6	15	67.7
ND: no data	BMDL: below method detection limit (5 ppb)				‡: Removed from study due to clogging			

4.8 Model F results

The sample size for model F included five model replicates. Experimental variables included, three test microbes (*E. faecalis, E. coli*, MS2), and seven microbe-seeded challenge water applications occurring at aging water volumes of 10L, 100-500L at 100-liter intervals, and 700L). De-chlorinated tap water with 1% pasteurized settled sewage was used as the aging and challenge water for the F model units. Regression charts and results tables for model F units can be seen Table 9 and figures 18-20.

Table 9: Test microbe LRVs and chemical concentrations or model F LifeStraws at each challenge interval for aging water

E. faecalis	**10**	**100**	**200**	**300**	**400**	**500**	**700**	Mean
F1	6.3	6.4	ND	ND	5.1	ND	ND	6.2
F2	6.8	>6.5	ND	ND	6	1.9	ND	>5.7
F3	>6.9	>6.5	ND	ND	5.8	1.9	ND	>5.6
F4	>6.9	>6.5	ND	ND	5.8	2.4	ND	>5.6
F5	>6.9	>6.5	ND	ND	5.9	2.6	ND	>5.7
***E. coli* B**								>5.8
F1	>6.6	>6.0	>6.1	>6.2	5.9	>6.6	>6.5	>6.3
F2	>6.6	>6.0	>6.1	>6.2	>6.7	>6.6	>6.5	>6.4
F3	>6.6	>6.0	>6.1	>6.2	>6.7	>6.6	>6.5	>6.4
F4	>6.6	>6.0	>6.1	>6.2	>6.7	>6.6	>6.5	>6.4
F5	>6.6	>6.0	>6.1	>6.2	6.3	>6.6	>6.5	>6.3
MS-2								>6.4
F1	1.7	1.3	0.3	1.2	1.1	1.3	0.7	1.1
F2	1.6	1.3	0.4	1.1	1.2	1.5	0.8	1.1
F3	1.7	1.3	0.3	1.1	1.2	1.5	0.9	1.1
F4	1.9	1.4	0.3	1.2	1.3	1.5	0.8	1.2
F5	1.8	1.4	0.4	1.4	1.2	1.6	0.9	1.2
Iodine (mg/L)								1.2
F1	BMDL	BMDL	BMDL	0.2	0.2	0.8	1	≤0.6
F2	BMDL	BMDL	BMDL	BMDL	BMDL	0.4	0.8	≤0.6
F3	BMDL	BMDL	BMDL	BMDL	BMDL	1	0.8	≤0.9
F4	BMDL	BMDL	BMDL	BMDL	0.4	1	1.5	≤1.0
F5	BMDL	BMDL	BMDL	0.2	BMDL	0.8	1	≤0.7
Iodide (mg/L)								≤0.7
F1	N/A	N/A	N/A	0.2	0.2	0.8	1	
F2	N/A		N/A	N/A	N/A	0.4	0.8	
F3	N/A	N/A	N/A	N/A	N/A	1	0.8	

F4	N/A	N/A	N/A	N/A	0.4	1	1.5	
F5	N/A	N/A	N/A	0.2	N/A	0.8	1	
Silver (ppb)								
F1	7	3	7	6	6	0		5.1
F2	6	2	5	2	3	0	5	3.3
F3	6	2	6	4	4	1	10	4.7
F4	5	2	3	3	1	0	2	2.3
F5	6	2	2	8	5	0	5	4.0
ND: no data	BMDL: below method detection limit (5 ppb)							3.9

From periodic challenges with test microbe-seeded water waters, the overall mean LRVs within model type F LifeStraws were 5.8 $\log_{10}$ (standard deviation ± 1.5 $\log_{10}$ for *E. faecalis*, and >6.4 $\log_{10}$ (standard deviation ± 0.4 $\log_{10}$ for *E. coli* B. For *E. coli* B, the lower detection limit of the assay method was met for the majority of LifeStraw effluent samples, and therefore, LRVs represent censored values and likely underestimate the true extent of reduction.. For MS2 coliphage, the mean overall LRV was 0.9 $\log_{10}$ (standard deviation = ±0.5 $\log_{10}$).

Challenge water effluent concentrations of iodine and iodide were below the minimum detection limit (BMDL 0.1 mg/l) in the majority of samples. Iodine detection occurred only in the later challenge water effluent samples, after 300 liters or more of aging water, and all of it was in the form of iodide. The maximum detected iodine level was 1.5 mg/L, the arithmetic mean of the measurable points was less than half the maximum (0.7mg/L). Because the mean iodine concentration does not include data pints below the detection limit (BMDL) the value is an overestimate of the actual mean iodine concentration. Mean values that are calculated from a data set that includes one or more points that were BMDL are indicated by a less-than-or-equal-to symbol.

There were detectable concentrations of silver in all LifeStraw effluent samples of challenge waters. The maximum detected silver concentration was 10 ppb, and the average

concentration was 3.9 ppb. Hence, all model F LifeStraws resulted in some silver release into challenge water effluents, although concentrations were far lower than the WHO guideline limit of 100 ppb.

4.8.1 Trends in LifeStraw microbial reductions over aging water volume

Based on linear regression analyses, the LRVs of all three test microbes in seeded challenge waters were consistent over the 700 L aging water volume of straw life design. However, slopes of fitted regression lines were not zero and LRVs were not always the same at the different challenge intervals for aging water volumes. For example, *E. faecalis* LRVs from seeded challenge water were consistently greater than 5.0 $\log_{10}$ for all aging water volumes through 400L, but declined to less than 3 $\log_{10}$ for all straws at 500L. Regression analysis for *E. faecalis* gave an R^2 of 0.6; however, there was no significant trend over aging. For *E. coli* and MS2 LRVs in challenge water effluents of over the 700L range of aging water volumes resulted in regression models that had wide 95% confidence bands ($R^2 = 0.2$ and 0.3 respectively) and no significant trends ($P > 0.5$).

Figure 18: Regression of *E. faecalis* LRV's over volume aged for model F

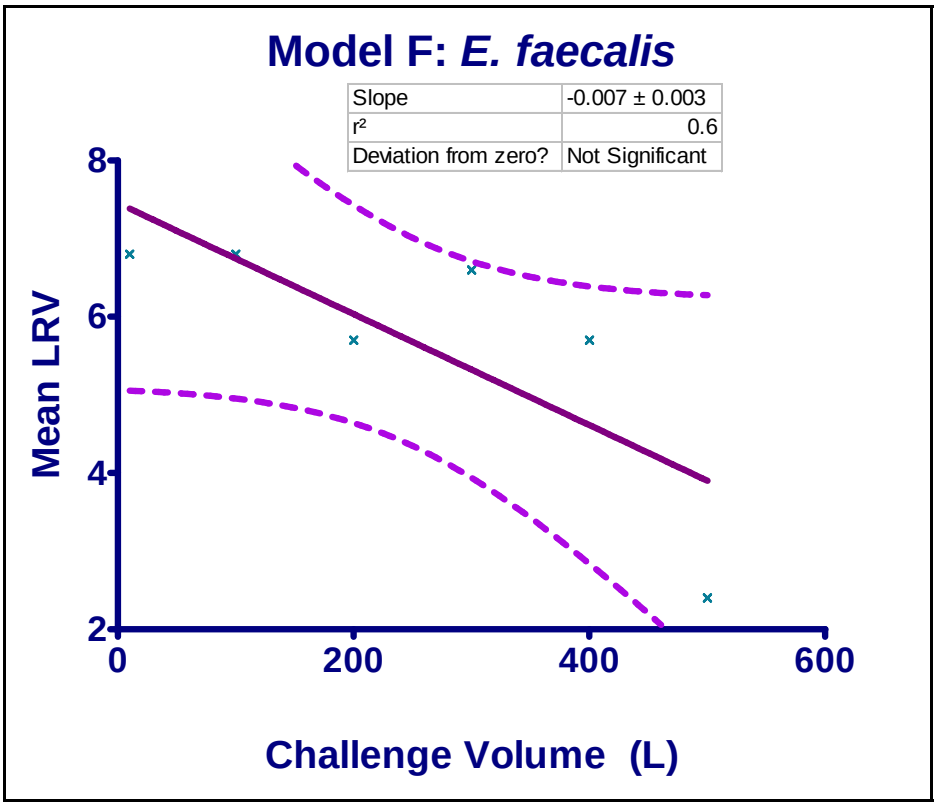


Figure 19: Regression of *E. coli* LRV's over volume aged for model F

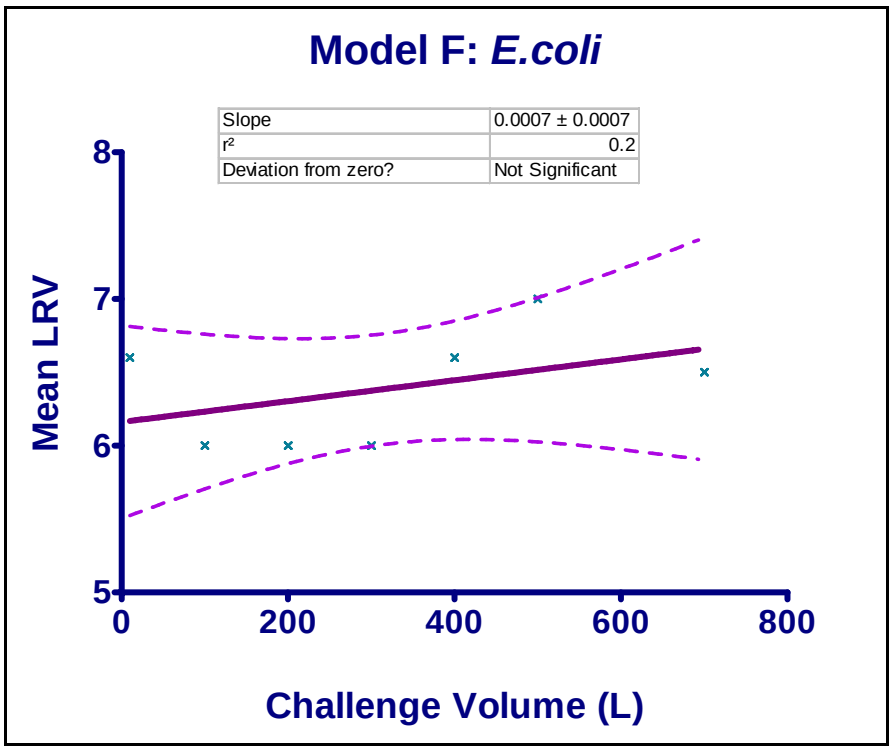
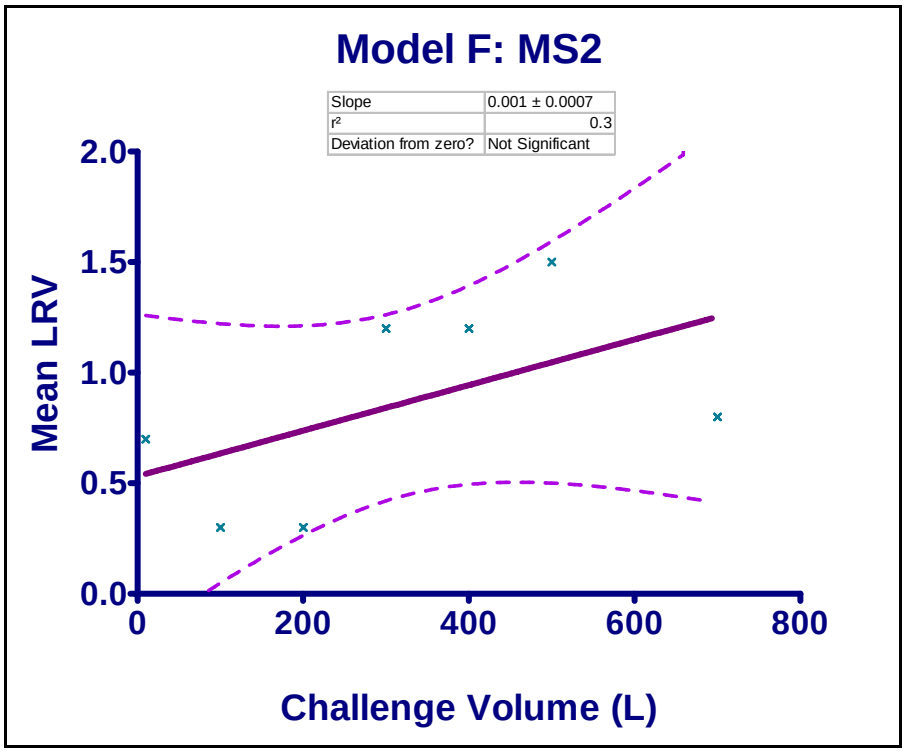


Figure 20: Regression of MS2 LRV's over volume aged for model F



4.9 Model NVO results

The sample size for model NVO LifeStraws included five model replicates. Experimental variables included two microbes, *E. faecalis* and MS2, and two seeded water challenges, after 10, and 100L of aging water. Aging water for the YAO models was de-chlorinated tap water only. The experimental design for the NVO model type included seeding challenge waters with *C. perfringens* as an indicator for spore-forming enteric pathogens and a surrogate for protozoan parasite cysts. However, there were insufficient data collected to present here because seeded influent challenge water titers were too low to calculate LRV's. Furthermore, the methods described in the previous chapter may have been incorrect and possibly was the source of the assay problems. Also, there were insufficient *E. faecalis* and MS2 data to perform robust statistical analyses for the microbial reduction

performance of the NVO model type. The data collected were used to compute overall mean LRVs, which are summarized in table 10.

Table 10: Test microbe LRVs and chemical concentrations for model NVO LifeStraws at two challenge interval for aging water

E. faecalis			
Model	10	100	Mean
NA	5.5	4.6	5.1
NB	>6.6	6.8	>6.7
NC	>6.6	6.1	6.3
ND	>6.6	>6.8	>6.7
NE	>6.6	6.3	>6.4
MS2			**6.2**
NA	0.8	0.9	0.8
NB	1.7	1.1	1.4
NC	1.2	0.9	1.0
ND	1.2	1.1	1.1
NE	0.8	1.2	1.0
Silver			**1.1**
NA	10	20	15
NB	15	5.0	10
NC	15	7.5	11
ND	10	10	10
NE	15	10	12
			11.8

The overall mean *E. faecalis* LRV from seeded challenge water by model NVO LifeStraws was 6.2 $\log_{10}$, with a corresponding standard deviation of ± 0.7. The standard deviation is low relative to the mean, which may reflect the large proportion of LRV data that were greater-than values. Greater-than values were based on LifeStraw challenge water effluent concentrations that were below the detection limits of the *E. faecalis* assays and therefore, were expressed as "less than" values.

Silver was detectable in all LifeStraw effluent samples from challenges after the available aging water intervals of 10 and 100 liters. Effluent concentrations of silver ranged from 5 to 20 ppb, with an average concentration of 12 ppb and a standard deviation of ±2.1. There were no detectable levels of iodine and iodide in the effluent water of the NVO model units throughout the challenge experiment.

4.10 Model YAO results

The sample size for model YAO LifeStraws included five model replicates. Experimental variables included two test microbes, *E. faecalis* and MS2 seeded into challenge waters applied delivered after 10 and 100L of applied aging water. Aging water for the YAO models was de-chlorinated tap water only. The experimental design for the NVO model type included seeding challenge waters with C. *perfringens* as an indicator for spore-forming enteric pathogens and a surrogate for protozoan parasite cysts. However, insufficient data were collected to present here because seeded influent challenge water titers were too low to calculate LRV's. Also, there were insufficient *E. faecalis* and MS2 data to perform robust statistical analyses for the microbial reduction performance of the YAO model type. The data collected were used to compute overall mean LRVs, which are summarized in table 11.

Table 11: Test microbe LRVs and chemical concentrations for model YAO LifeStraws at two challenge interval for aging water

E. faecalis			
Model	10	100	Mean
YF	>6.6	6.5	>6.5
YG	>6.6	6.2	>6.4
YH	>6.6	>6.8	>6.7
YI	>6.6	6.1	>6.3
YJ	>6.6	>6.8	>6.7
MS-2			**6.5**
YF	0.6	1.0	0.8
YG	0.4	0.9	0.6
YH	0.3	ND	NA
YI	0.3	0.6	0.4
YJ	0.7	0.8	0.7
Silver			**0.6**
YF	15	10	12.5
YG	15	15	15
YH	15	20	17.5
YI	15	10	12.5
YJ	15	10	12.5
			14

The overall mean LRV for *E. faecalis* from seeded challenge waters by model type YAO LifeStraws was 6.5 $\log_{10}$, with a standard deviation of ± 0.2. The standard deviation is low relative to the mean, which may reflect the large proportion of LRV data that were "greater than" values. These "greater than" values were based on LifeStraw challenge water effluent concentrations that were below the detection limits of the *E. faecalis* assays and therefore, were expressed as "less than" values.

Silver was detectable in all LifeStraw effluent samples from challenges after the available aging water intervals of 10 and 100 liters. Effluent concentrations of silver ranged from 10 to 20 ppb, with an average concentration of 14 ppb and a standard deviation of ±2.2.

There were no detectable levels of iodine and iodide in the effluent water of the YAO model units throughout the challenge experiment.

4.11 Models C1-C5 results

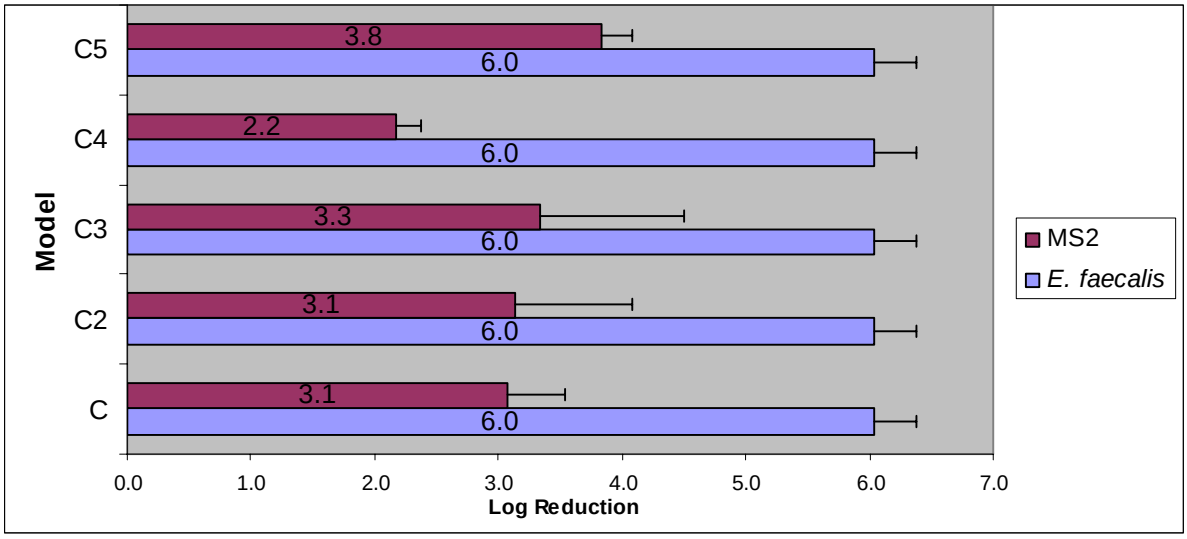
There were five different C model LifeStraw types tested; each represented by one unit. The performance data for C model LifeStraws provides an opportunity to make general statements about their performance with respect to microbial reductions, effects of aging, and concentrations of key leachable chemicals in the effluent. However, the lack of replicates within the C models does not allow for robust statistical analyses based on data from multiple units of the same type. Experimental variables included two test microbes, *E. faecalis* and MS2, and three seeded challenge water intervals after aging water volumes of 18L, 111L, and 312 L. At 312L of aging water, the test Lifestraws had clogged so that they would not maintain a flow of 150ml/min for more than a few minutes after backwashing. The C model experiments were terminated after three challenges due to LifeStraw clogging. The performance data collected from these three challenges are represented as overall mean values in table 11 and figure 21.

Table 11: Test microbe LRVs model C LifeStraws at three challenge interval for aging water

E. faecalis				
Model	18L	111L	312L	Mean LRV
C	>6.1	>6.4	>5.6†	>6.0†
C2	>6.1	>6.4	>5.6†	>6.0†
C3	>6.1	>6.4	>5.6†	>6.0†
C4	>6.1	>6.4	>5.6†	>6.0†
C5	>6.1	>6.4	>5.6†	>6.0†
MS2				
C	3.6	2.9	2.7	3.1
C2	4.1	3.1	2.2	3.1
C3	3.8	>4.2	2	>3.3
C4	2.4	2.1	2	2.2
C5	3.6	4.1	3.8	3.8

E. faecalis in challenge water was reduced to the same extent by all five of the C models at each challenge water interval, with LRVs of >6.1, >6.4 and >5.6 $\log_{10}$ (at aging water volume intervals of 18, 111 and 312 liters, respectively). All of the LRVs for *E. faecalis* are "greater than" values based on LifeStraw challenge water effluent concentrations that were below the detection limits of the *E. faecalis* assays and therefore, were expressed as "less than" values. Mean MS2 LRVs from seeded challenge waters ranged from 2.2 to 3.8 $\log_{10}$, depending on the C LifeStraw model tested. Overall, LifeStraw model C5 achieved the consistently highest MS2 LRVs over the 3 aging water challenge interval, with reductions of 3.6, 4.1 and 3.8 $\log_{10}$.

Figure 21: Overall mean LRV for models C-C5: MS2 and *E. faecalis* with (error bars)



Chemical analyses for the C model LifeStraws were completed separately from challenge procedures (see table 12). Chemical tests on effluent waters of Model C LifeStraws were done six separate occasions (from June 19th -26th, 2007). C model LifeStraw were challenged and aged with DTW only. Silver was present at detectable levels in effluents of 4 of 5 C model LifeStraws tested in all or some daily effluent samples. Model C4 had the consistently highest silver concentrations in effluent waters, at 35 to 40 ppb (mean = 39 ppb). Models C1 and C5 had lower silver concentrations with averages of 18 and 13 ppb, respectively, Model C2 had only 5 ppb silver in one sample and was BMDL in the other four. Model C2 was BMDL for all samples. Iodine was not detected in effluents of 3 model C LifeStraws (C1, C2 and C4) and present at only low concentrations in only one of 5 samples tested for models C3 and C5 (1.6 and 0.4 mg/l, respectively).

Table 12: Chemical concentrations for model C LifeStraws at six different challenge intervals over 8-days

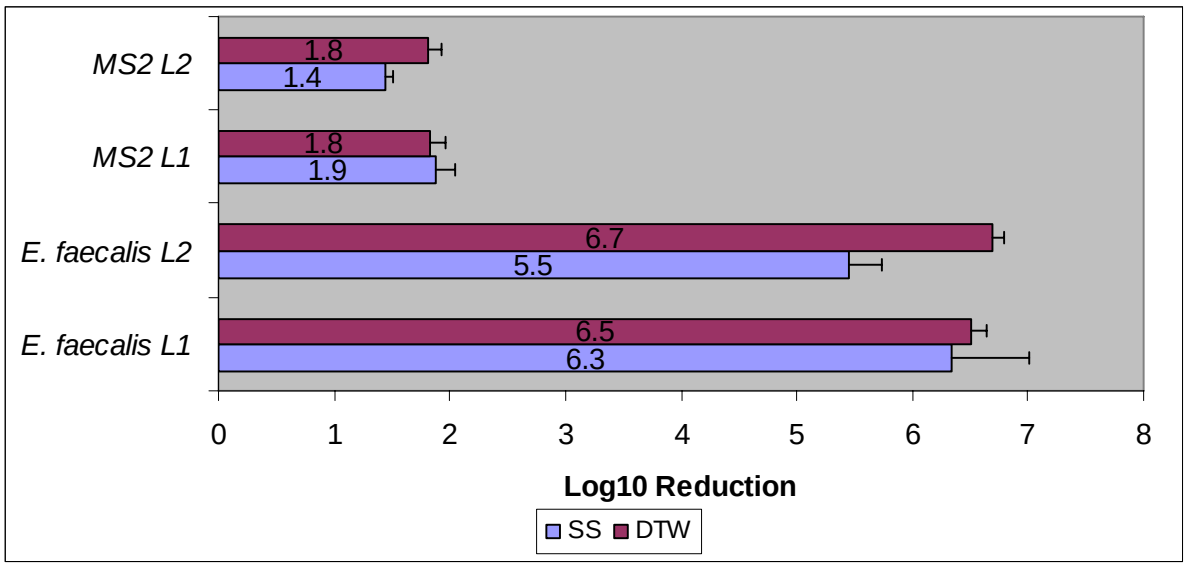
Iodine (mg/L)	6.19.07	6.20.07	6.21.07	6.22.07	6.25.07	6.26.07
C	ND	BMDL	BMDL	BMDL	BMDL	BMDL
C2	ND	BMDL	BMDL	BMDL	BMDL	BMDL
C3	ND	1.6	BMDL	BMDL	BMDL	BMDL
C4	ND	BMDL	BMDL	BMDL	BMDL	BMDL
C5	ND	0.4	BMDL	BMDL	BMDL	BMDL
Silver (ppb)						
C	ND	20.0	15.0	18.0	20.0	15.0
C2	ND	BMDL	BMDL	BMDL	BMDL	BMDL
C3	ND	5.0	BMDL	BMDL	BMDL	BMDL
C4	ND	40.0	40.0	40.0	35.0	40.0
C5	ND	BMDL	21.0	21.0	15.0	8.0

4.12 Trends in LifeStraw performance according to test water quality

Two different test waters were used for LifeStraw testing, dechlorinated tap water and dechlorinated tap water supplemented with 1% pasteurized settled sewage. The latter water type was intended to challenge the ability of the straws to remain unclogged over increasing water volume tested, up to 700 liters, and to determine if there was an affect on the ability of the LifeStraw models to reduce test microbes and prevent leaching of iodine and silver. Only two of the LifeStraw model types, L1 and L2, had adequate performance data for both types of test water to make a statistical comparison of performance. As shown in figure 22, model type L1 $\log_{10}$ reductions of MS2 were not significantly different between the two test waters. Similarly, model L1 $\log_{10}$ reductions of *E. faecalis* were not significantly different between the two water types. However, for both MS2 and *E. faecalis*, model type L2 $\log_{10}$ reductions were significantly lower (P= 0.0034 and 0.0049 respectively) when using DTW with 1% settled sewage compared to only DTW. Because the two comparable model types did not have the same patterns of microbial reductions in relation to water type, and there were only

a limited amount of data to compare their performance in the two water types, a reliable statement cannot be made about the influence of water type on the ability of the LifeStraw to reduce test microbe concentrations.

Figure 22: Model L1 and L2: Water type comparison using ANOVA (with error bars)



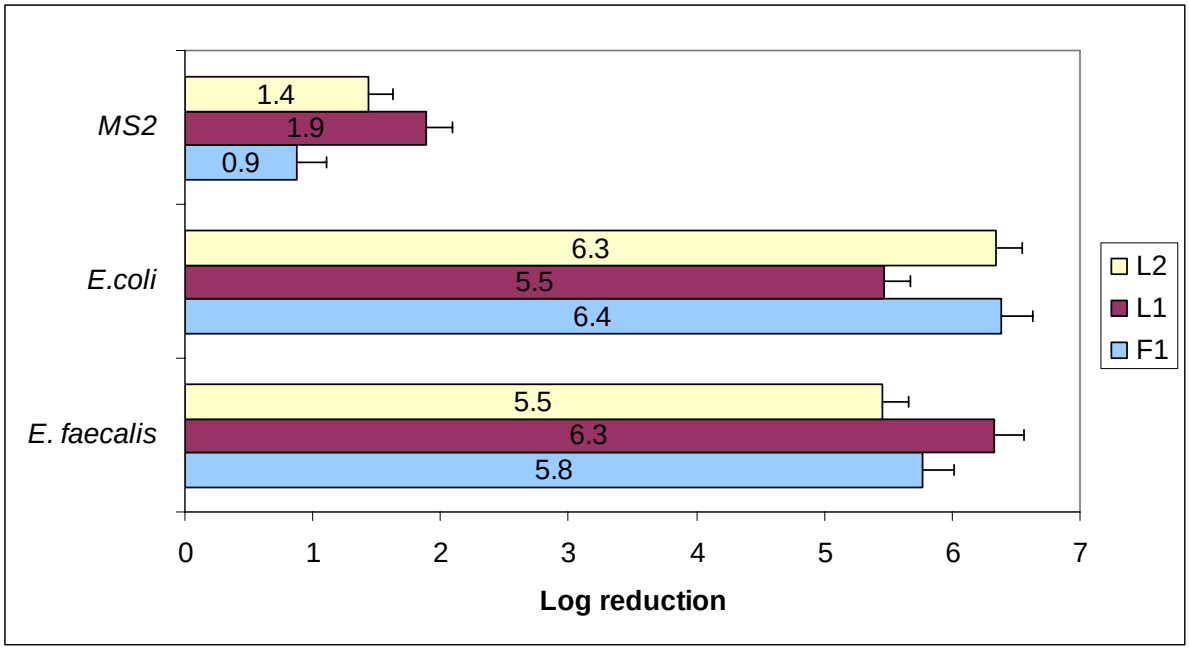
4.13 Trends in LifeStraw performance among different models

Testing of models L1, F1 and L2 provided enough performance data to compare LRV's for test microbes among the three model types using one-way ANOVA (see figure 23) The data used to compare among models excluded units that were aged with DTW only. For reductions of coliphage MS2, model type L1 had significantly higher LRV's than models L2 and F1. Model L1 MS2 reduction (1.8 $\log_{10}$) was ~0.4 $\log_{10}$ higher than model L2 (1.4 $\log_{10}$), and ~0.9 $\log_{10}$ higher than Model F1 (0.8 $\log_{10}$). ANOVA testing found that all three model types were significantly different from each other (P=0.0016). Model L1 also had higher mean reductions of *E. faecalis* than L2 and F1 model types. The mean reduction for L1 (6.3 $\log_{10}$) was 0.5 $\log_{10}$ higher than F1 (5.8 $\log_{10}$) and 0.8 $\log_{10}$ higher than L2 (5.5

log_{10}). However, ANOVA testing did not find a statistically significant difference between the model types for *E. faecalis.*

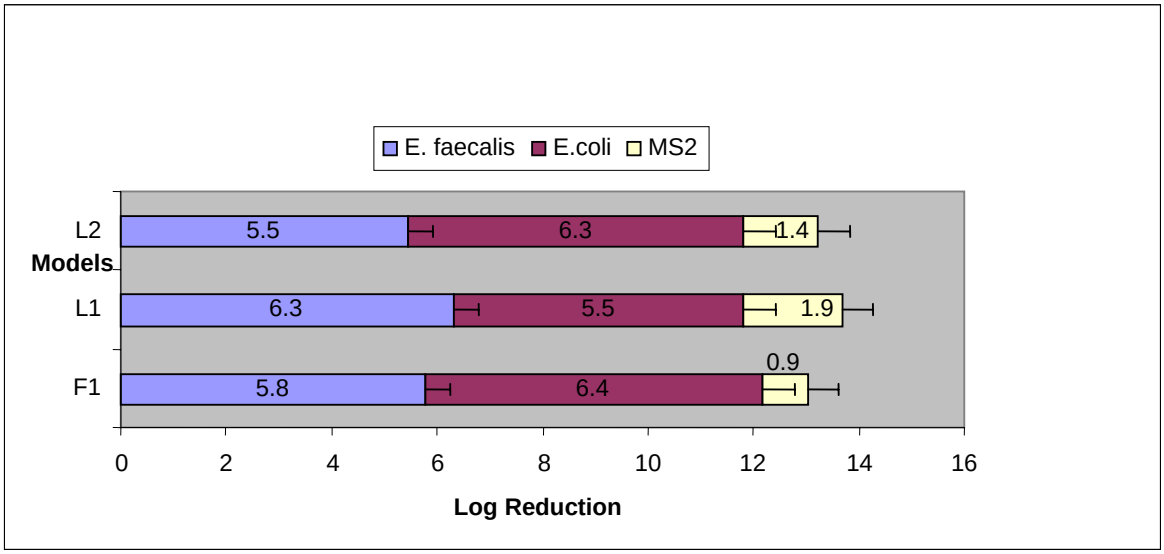
As shown in Figure 23, comparison of mean reductions of E. coli found F1 and L2 to be insignificantly different from each other (6.39 log_{10} and 6.34 log_{10} respectively), while model type L1 had a significantly (P=0.0016) lower reduction of E. coli (5.5 log_{10}).

Figure 23: ANOVA means of models L1, L2, F (SS): with error bars



To make an overall comparison among model types the mean reduction values of the three tested microbes were summed (figure 24). The overall microbe reduction ability of L1, L2 and F1 model types does not differ significantly. Summed reductions ranged from 13.0 (F1) to 13.7 (L1) for the three models, and mean standard error ranged from 0.46 (F1) to 0.63 (L1).

<u>Figure 24</u>: Overall microbe reduction ability by LSM: with (error bars)



DISCUSSION

5.1 Preamble

The LifeStraw experiments of this study provided considerable insight into all three of the original aims. These aims were 1) to determine the ability of Lifestraws to reduce concentrations of select test microbes in respect to target reductions, 2) to challenge the physical ability of the LifeStraw models tested in regards to clogging over volume aged, and 3) to assess the degree of disinfectant concentrations in the effluent water with respect to target levels set to protect consumer health. Results clearly show that, of the models tested, none were able to reduce the test virus MS2 coliphage, at or near the target 4 $\log_{10}$ reduction level set by the US EPA and NSF-International. However, both of the test bacteria, *E. faecalis* and *E. coli* were reduced at or near the 6 $\log_{10}$ target reduction goal by most of the LifeStraw models tested. Concentrations of iodine in the effluent water were consistently below the guidelines set forth by the EPA. Silver concentrations in the effluent water varied widely among model type and challenge series, and were occasionally above WHO guidelines and EPA standards for drinking water. The LifeStraw experiments showed that some models were unable to maintain an acceptable water flow rate at a practical target use pressure for the life of the device. Other performance-related findings from the LifeStraw experiments included temporal patterns in microbial reduction with increased volume of aging water, the effect of aging water type, and comparative reductions of morphologically similar test microbes. Each of these findings will be discussed by first, looking at the

strength and variability of the supporting results, comparing the results to previous similar works, and discussion of the greater implications of the findings.

Log_{10} reductions are reported for each challenge interval of water received and as total log_{10} reduction over the entire 700 L volume of water received by each LifeStraw. Results that achieved the target reduction in the presence of 1% pasteurized settled sewage in the test water are highly encouraging, as this quality of water more realistically models conditions found in feces-contaminated, but "clear" (low turbidity) waters in developing countries.

5.2 Lifetime microbial reductions

The most encouraging results from the Lifestraw experiments were the lifetime LRV's for indicator bacteria. Both *E. faecalis* and *E. coli* were consistently reduced between 5 and 6 log_{10} during their 700-liter volume lifetime across all Lifestraw model types. For the LifeStraw models that did not meet the 6 log_{10} target but came close to it, it is important to consider that the lifetime mean LRV's were calculated using greater-than values (minimum threshold values) which represent the lowest possible log_{10} reduction. In these cases the characteristics of the model may have less to do with the ability to meet the target LRV than variability in the lower detection limit of the bacteria assay. If necessary, further laboratory research could be done to determine the "real", uncensored LRV for the two test bacteria.

Although there are few studies that are comparable to the LifeStraw experimental design, the LRV's found for bacteria when testing treatment devices with similar disinfectant components reported some similar results. However, the LRV's for the LifeStraw were significantly higher than reported for some other iodinated resin purifiers, but not others. (Clasen & Menon, 2007; Schlosser et al., 2001). Two iodinated resin purifiers tested on clear

waters like those of this study achieved viable bacteria $\log_{10}$ reductions of only 2.4, 3.0 and 1.3 respectively. (Schlosser et al., 2001) The implication of the findings from the LifeStraw experiments is that the LifeStraw could be a very effective tool for reducing burden of diarrheal disease from waterborne, enteric, bacterial pathogens.

Although the LifeStraw was shown to be effective for reducing indicator bacteria, results clearly show that all models of the LifeStraw tested had lifetime LRV's of less than half the target 4 $\log_{10}$ reduction of the test virus, MS2 coliphage. Confidence in this finding is strong for two reasons. (1) The mean lifetime LRV was based on measurable deferens in $\log_{10}$ concentrations in seeded challenge and Lifestraw effluent waters and not on greater-than values. (2) MS2 reductions in the effluent were similar across model types, challenge series and water type. Although model L1 had the highest lifetime LRV for MS2 of 1.8 $\log_{10}$, the lifetime LRVs for models L2 and F were within a $\log_{10}$ value of model L1. Although this variation in LRV was significant, LRVs of all three Lifestraw models were significantly below the 4 $\log_{10}$ reduction target. Therefore, other performance indicators would need to be used to determine the most effective model type for overall performance.

Previous research also reports LRV's of MS2 between 1 $\log_{10}$ and 3 $\log_{10}$ for iodinated resin treatment devices. Water treatment devices that use chlorine dioxide as a disinfectant have shown much higher LRV's for MS2 coliphage. (Clasen & Menon, 2007; Jensen et al., 2003; Souter et al., 2003) The relatively low reductions of MS2 coliphage found in the LifeStraw experiments, compared to the 4 $\log_{10}$ performance target of certifying entities, indicate that the LifeStraw will not reduce disease burdens from important viral pathogens like enteroviruses (poliovirus, coxsackievirus, and echovirus) and rotaviruses to the desired level. Because the LifeStraw has high bacterial reduction abilities, the device

could be effectively used for reduction of enteric bacteria. To improve on viral control, it could be used in combination with one or more POU water treatment interventions that are effective against viruses.

5.3 Iodine and silver concentrations in effluent water

There is no established target maximum iodine concentration in the effluent water of the LifeStraws. The majority of the results found no detectable levels of iodine or iodide in the effluent water. The few detectable levels of iodine ranged from 0.2 mg/L (the detection limit) to 1.6 mg/L in a C-series unit. Of the models tested, none indicated a significant change in iodine levels over volume aged or between water types. The low levels of iodine detected in the effluent water of some LifeStraw models was well below WHO guideline levels for iodine allowed in intermittent use drinking water. Iodine is an essential element for the synthesis of thyroid hormones, and in many parts of the world, there are dietary deficiencies in iodine. Estimates of the dietary requirement for adult humans range from 150 to 200 μg/day. The amount of iodine from drinking water exposure at the highest level detected throughout the experiments, with consumption of two liters per day, would amount to 3,200 μg iodine per day. This concentration of iodine is about 15-20 times above the recommended daily intake. However, the average concentration of iodine was well below this level and mostly below the method detection limit of 0.2 mg/l, which is about the recommended daily intake of iodine. Therefore, over the course of daily use for up to nearly a year (700 liters of water at 2 liters per day), the overall iodine contribution of the Lifestraw to the 150-200 μg/day dietary requirement would be considered minor and would not constitute a health risk. A review of research concerning health affects of continuous intake

of iodine treated water suggests that 2 mg/day of iodine is recommended, and higher doses (less than 4 mg/day) have not shown any adverse clinical affects. (Backer, 2000)

Silver was present more consistently than iodine in the effluent water of the LifeStraws. Silver concentration in effluent water ranged from ≤ 5 ppb (the detection limit) up to 200 ppb for some of the L2 model units. Measurable effluent silver concentrations of all five LifeStraw models, averaged over the lifetime of the device, are below the WHO guideline value and the US EPA maximum contaminant level (MCL) of 100 ppb. Although models L1 and L2 had several effluent concentrations of silver that were 50% -100% higher than the MCL, the levels do not constitute a health risk because adverse health effects from silver require long-term (decades) exposure to levels far higher than those detected in the effluents produced by the LifeStraw models tested in this study (WHO, 2006; (M. D. Sobsey, 2007). There were no significant changes in trends of silver concentration in the effluent over aging volume for any of the five model types tested.

5.4 Temporal patterns of microbial reductions by LifeStraws over water aging volume

The trends in Lifestraw performance over aging water volume are useful to consider in determining the effectiveness of the LifeStraw. Two factors negatively influenced the ability to describe trends over water volume aged throughout the LifeStraw models. (1) When the lower detection limit of the bacterial assay was consistently exceeded in effluent water the resulting microbe reductions were shown as greater–than-values making it impossible to observe actual performance trends in LRV over aging water volume. (2) Missing data from specific challenges was also a problem, but in only one or two cases.

5.5 Model L1

By regression analysis of model L1 LRV's for *E. faecalis* and MS2 coliphage, there was no significant change in performance over the volume of aging water. The "no change" trend over volume of aging indicates that with prolonged contact with flowing water over time, the microbiocidal effectiveness of iodinated resin disinfectant within the LifeStraw was not compromised. Another expected trend of the LifeStraw was an observed decrease in effectiveness over time as the availability of the disinfecting chemical was either (1) used up or (2) the active mechanism was hindered by a biological or chemical process (e.g. biofilm growth on resin beads). In the case of LRV's of *E. coli* for model L1 units, regression analysis documented a significant increase in LRV's over the volume aged. This trend was unusual and unexpected for two reasons; (1) the disinfectant mechanism of the LifeStraw was not expected to increase in effectiveness over volume aged, and (2) if the disinfectant ability of the LifeStraw did increase over aging, *E. faecalis* and *E. coli* would likely give a similar response. The apparent increase in LRV of E. coli probably was not due to a change in actual performance but rather the result of having improved the lower detection limit of the microbial assay methods in the later challenges done as the volume of aging water increased. Hence, the observed changes in LRV over water volume of aging were an artifact created by changing detection limits of microbial assays and not an actual change attributable to LifeStraw performance.

5.6 Model L2

By regression analysis of the LRVs for model L2 units there was a significant decrease in *E. faecalis* reduction over volume of aging water. The coefficient of determination for the regression of the LRV's for *E. faecalis* was strong and indicated confidence in the decreasing LRV trend. Although there were nine identical model replicates

tested, there was observed variation between the units tested during the L-series trials and those tested in the F-series trails. The F-series units provided more definitive performance data because there were fewer LRV's represented by greater-than values. The L2 units tested in the L-series trials reached the LRV greater than detection limits due to the below detection limit results of effluent water assays of the majority of challenge points for *E. faecalis*. LRV's for *E. coli* did not show a change in trend in LRV performance with increasing volume of aging water. Most of the data points for the LRV for all nine replicates of LifeStraws were above the detection limit, as a result of below detection limit results for assays of the effluent samples. Model L2 reductions of *E. coli* were similar to those of model L1 in that observations of changes in performance trend over increasing aging water volume only reflect detection limits and not uncensored $\log_{10}$ reduction values. By regression analysis of the MS2 LRVs there was no significant trend in change of performance over volume of aging water. Confidence in the "no trend" observation for MS2 reductions was better than for *E. coli* and *E. faecalis* because all of the data points represent actual LRVs and not "greater than" values.. Compared to those for bacteria, MS2 reductions were low overall. The regression analysis results imply that disinfectant ability of the LifeStraw did not change over its lifetime.

For all three microbes tested on L2 units, there were noticeable differences in LRVs between the L-series replicates and the F series. However, much of this is attributable to variability in lower detection limits of effluent assays of test bacteria and not in actual performance. However, LRV's for *E. faecalis* varied significantly between series replicates and the magnitude of LRV detection limits was not a factor. In this case, differences between LifeStraw series were likely a result of the water type used. The L-series used de-

chlorinated tap water while the F-series used de-chlorinated tap water with 1% settled sewage.

5.7 Models L3-L6

Models L3-L6 LifeStraws have similar results to those of model L1 and L2. Models L3 and L4 experienced premature clogging after only 200L of aging water. Therefore, available data were insufficient for all three test microbes to examine for changes in performance trends over aging water volume. LRVs of *E. faecalis* consistently met the target 6 $\log_{10}$ reduction as greater than values due to effluent water microbe concentrations below the detection limits of the assay. The mean lifetime $\log_{10}$ microbe reductions were low, because, the value of the greater than LRV was small, due to an inadequate detection limit value of only 2.8 $\log_{10}$ at the 200L challenge point. LRV for MS2 coliphage were similar to those of L1 and L2 model LifeStraws with lifetime mean MS2 reductions ranging from 2.2 $\log_{10}$ to 1.6 $\log_{10}$.

5.8 Model F

By regression analysis of the model F replicates, LRVs for all three microbes tested did not indicate significant changes in performance over increasing aging water volume. However, the coefficients of determination were relatively low for all three microbes (R^2 ranged from 0.2-0.6) indicating that confidence in the regression lines was relatively low. LRV's for *E. faecalis* declined in the 500L challenge compared to earlier challenges. However, a significant downward trend could not be established because the 700L challenge did not provide usable data. The majority of LRVs for *E. coli* in the model L2 units were greater than values because effluent microbe concentrations exceeded lower detection limits of the assays. However, the greater than LRVs were all at or above 6 $\log_{10}$, thus meeting or

exceeding the reduction target of certifying entities such as US EPA and NSF-International. MS2 LRVs for model L2 LifeStraws did not show a significant change in magnitude, as documented by regression analysis. However, it was clear that the LRVs first three challenge points were considerably lower than those of challenges 4, 5 and 6.

5.9 Models NVO and YAO

The NVO and YAO LifeStraw model types were tested in the same series of experiments. All ten straws in the series (five NVO units and five YAO units) experienced premature clogging with 100L to 200L of aging water. Testing of the units was discontinued because the LifeStraws were not capable of maintaining a consistent flow of 150 ml/min. The NVO and YAO series experiment used de-chlorinated tap water with 1% settled sewage as the aging water. Although results using this aging water more accurately portray real-use conditions, its use was likely a contributing factor in LifeStraw clogging.

Examining performance trends over aging water volume processed for the NVO and YAO model units was not possible because of the lack of sufficient data to perform regression analysis. Without the ability to observe performance trends over aging water` volume for the NVO and YAO models, the best insight into the microbial reduction performance of the models was the mean lifetime $\log_{10}$ reduction. Some LRVs of *E. faecalis* for model NVO units were greater-than-values because effluent concentration of microbes were below the detection limits of the assays. However, the detection limits were above the target 6 $\log_{10}$ reduction. From the limited amount of data collected, model NVO appears to have the ability to achieve enteric bacteria reductions above the target reduction of 6 log10. Reductions of MS2 coliphage in both NVO and YAO models were well below the 4 $\log_{10}$ target, at only 1.1 log10 and 0.6 log10 respectively. These reductions are similar to those of

MS2 in other LifeStraw models. Given the generally similar MS2 $\log_{10}$ reductions, it is not possible to distinguish performance differences among the different models based this test microbe. However, the MS2 performance data are important for evaluating the, overall microbial performance of the different LifeStraw models and their design features.

5.10 Models C1-C5

In the C series experiments five model types (C1-C5) were tested. All five units in this series experienced premature clogging at just over 300L of aging water processed. LRVs of E. *faecalis* for the five models were greater than values because effluent microbe concentrations exceeded the lower detection limit of the assays at all challenge points. Although the C models of LifeStraws exceeded the 6 $\log_{10}$ target reduction for bacteria, their tendency for premature clogging made them less effective than the L and F models. MS2 reductions by the C model LifeStraws were higher than those of the other LifeStraw model types testing, ranging from 2.2 log10 to 3.8 log10. Although these MS2 reductions are encouraging and better than other models tested, they do not meet the 4 $\log_{10}$ target reduction of the US EPA and NSF-International. Furthermore, conclusive statements of performance cannot be made due to the small sample size from limited testing.

5.11 Comparison of LifeStraw performance according to water types used for aging

Throughout the LifeStraw experiments two types of water were used for aging. Dechlorinated tap water was used to create aging water low in constituents that influence microbial reductions, clogging, or chemical interaction with the disinfectants. The second water type included 1% pasteurized settled sewage with dechlorinated tap water to simulate slightly turbid but fecally contaminated water. Models L1 and L2 were the only two LifeStraws tested using both water types. Cross model comparison on the effect of aging

water composition is not appropriate because of potential differences in LRVs due to LifeStraw design differences. Model L2 had the largest sample size to compare water types; units L3-L5 were aged with de-chlorinated tap water while L7 and F6-F10 were aged with de-chlorinated tap water with 1% settled sewage water. Of the three L1 model replicates, L1 and L2 were aged with de-chlorinated tap water and L10 was aged with de-chlorinated tap water with 1% settled sewage. Results from ANOVAs are unclear. Statistical comparisons for Model L2 show significantly lower reductions of MS2 and *E. faecalis* in the presence of de-chlorinated tap water with 1% settled sewage compared to dechlorinated tap water alone as aging water. However, model L1 does not show significant differences in microbe reductions between the two water types. The largest difference in LRVs between water types was 1.2 $\log_{10}$ between least square mean LRVs of *E. faecalis* in LifeStraw model L2. Other evidence supports this statistical difference in *E. faecalis* reduction between water types treated by model L2. Figure 25 illustrates a decreasing *E. faecalis* reduction over increasing aging water volume for units aged with de-chlorinated tap water with 1% settled sewage when compared to the de-chlorinated tap water aged units. Although this evidence suggests a possible relationship between water type used for aging and LRVs, there is not a consistent performance trend over a sufficient sample size for this effect to be considered conclusive.

5.12 Sources of variability and uncertainty

A large source of uncertainty throughout the LifeStraw experiments came from the inability of the assay method for *E. faecalis* and *E. coli* to achieve detectable levels of remaining microbes in effluent waters. More than 70% of the LRV's of *E. coli* and *E. faecalis* across all models were greater than values because effluent water has bacteria concentrations below the detection limits of the assay. There are two major implications of

the large proportion of greater-than values for LRVs: (1) an inability to observe changes in LRV trends over aging, and (2) an underestimation of the LRVs for the two test bacteria. Fortunately, more often than not the lower detection limit of the effluent assay method and the bacteria concentration in the seeded test water were great enough to document LRVs above the performance target 6 $\log_{10}$ reduction of certification entities such as US EPA and . This allows for confidence in statements about the ability of the LifeStraw to reduce bacteria concentrations sufficiently to meet such performance target reductions.

Another source of uncertainty relating to the large number of data points that exceeded the upper detection limit of LRVs was the inaccuracy of averaging the detection limit LRVs. To get lifetime mean LRVs for a unit that has greater than LRVs, only a minimum threshold value of performance was established. Averaging minimum values over the water volume lifetime of a unit and then averaging the lifetime performance value of the unit across all units in the model type has the potential to reflect a reduction capability that is much lower than the actual value. The uncertainty that is created is the extent to which the real reduction is represented by the averaged greater than threshold value.

Two minor causes of missing data were from clogging of the LifeStraws and non-measurable results from the assay methods. Clogging was a factor that was a LifeStraw performance criterion for the experiments. Although it caused gaps in data collection, it provided insight into the overarching goal of selecting the LifeStraw model with the best performance. Lack of data due to clogging significantly impacted the NVO, YAO, and C models because all of the replicate units clogged at approximately the same volume aged; in these cases, clogging lead to premature termination of the challenge experiments.

A variety of experimental variables confounded the ability to clearly interpret the mechanisms of relationships, trends and generalizations in the datasets. Log_{10} reduction values for the indicator microbes had the potential to be influenced by the model type, aging water type, experimental series, the volume of aging water, and the assay method used. Accounting for these variables in the data analysis sub-divided already small sample sizes into even smaller subgroups of samples (e.g. LRV's of model x, water type y, microbe z, in series w). In an effort to obtain sufficient sample sizes for statistical analyses, changes in assay methods and variability between series were assumed to have a negligible impact on overall LRVs and so the data for them were combined.

The final source of variability and uncertainty to be mentioned was the lack of replicate units for certain LifeStraw models. Low replicate numbers increased variability and uncertainty in the results simply because there were less data with which to examine trends. The C models as well as some of the LF07 models (models L3-L5) had only one test unit per model. In the best case, one unit could provide seven LRVs for each test microbe throughout the lifetime or repeated performance observations. Because of low numbers of replicates, natural or uncontrollable variability and variability from assay methods and experimental design have a stronger influence than they would with larger numbers of replicates.

CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

1.1 billion people lack access to improved drinking water supply. (WHO, 2007) Those without access to safe water also represent the majority (88%) of the 4 billion cases of diarrhea each year. (WHO, 2007) The burden of diarrheal disease looms heaviest on those in poverty. Without access to clean water and sanitation the ability to work, live, and raise a family are ever-complicated by costs of medical care to treat waterborne diseases, the time and effort to collect and store water, and the time and money needed to care for disease burdened children. The LifeStraw has been designed as an answer to the need for preventative interventions for diarrheal disease. The LifeStraw is a relatively low-cost, easy-to-use POU water treatment device that has the potential to affect an enormous amount of change on the burden from waterborne disease in developing countries and after large-scale disasters. This study has tested the performance of the LifeStraw by three measures; its ability to reduce test microbes to target levels, the lifetime of the straw in regards to clogging, and the quality of the effluent water in respect to concentrations of chemical disinfectant. The LifeStraw studies included 14 model types tested over four experimental series. Key conclusions from the study are summarized below.

6.2 Conclusions

1 Most LifeStraw models tested have shown reductions of gram-positive indicator bacteria (E. faecalis) at or above the target reduction of 6 log10 (≥99.9999%).

2 Most LifeStraw models tested have shown reductions of gram-negative indicator bacteria (E. coli) at or above the target reduction of 6 log10 (≥99.9999%). Models that did not reach the target value were restricted by the detection limit of the assay method; the real reduction capability was inconclusive.

3 None of the LifeStraw models tested reached the target reduction (4 log10; 99.99%) of MS2 coliphage. Most reductions of MS2 coliphage were at or below 2 log10 (≤99.0%).

4 All of the F models and most of the L models maintained a consistent inflow rate of 150 ml/min for the manufacturer's intended product life of ≥700L of treated water. Models L5, L6, NVO, YAO and C1-C5 were not able to maintain a consistent inflow rate of 150 ml/min for the manufacturer's intended product life of ≥700L when back-flushed at regular intervals (every 9L).

5 In LifeStraw models tested, concentrations of iodine in the effluent water were consistently below the maximum suggested level for repeated consumption (2 mg/day). The majority of the LifeStraw models had no detectable levels of iodine throughout their intended lifetime.

6 Most LifeStraw models had measurable concentrations of silver in the effluent water. There were some measurements that exceeded the suggested maximum silver concentration (100 ppb/day), however occasional higher silver levels (100 to 200 ppb) observed in effluents of model L1 and L2 LifeStraws do not constitute a health risk from the treated water. This is because adverse health effects from silver require long-term (decades) exposure to levels far higher than those detected in this study.

7 Reductions of both gram-negative and gram-positive bacteria are likely to be significantly underestimated because of the inevitable reflection of assay detection limits in many of the reduction values. Although reduction values that are impacted by a detection limit/s are indicated by a greater-than value, the "real" reduction capability is ambiguous.

8 Both trends in microbial reduction over time as well as trends between aging water types were inconsistent across model types. However, within some LifeStraw models significant trends were established.

8.1 Model L2 showed decreasing reductions of E. faecalis over aging with de-chlorinated tap water with 1% pasteurized settled sewage, but no decrease in reduction capability when aged with de-chlorinated tap water only.

9 Confidence in determining trends over aging as well as in overall reductions of *E. coli* and *E. faecalis* were confounded by the detection limits of the assay method.

6.3 Recommendations

- The low number of replicates in the LifeStraw experiments allowed for a larger number of model types to be tested in a relatively short period of time. This is a successful strategy for "weeding out" ineffective models. However, a larger sample size is necessary to create a robust data set that would facilitate valid conclusions. Future LifeStraw testing should include at least ten and ideally thirty replicates of each model[1]. Replicates should be tested in at least three separate series with "blinded" laboratory technicians. Spreading the replicates across multiple series and blinding technicians will allow for an unbiased dataset where effect of series on the results can be quantified.

- Although silver concentrations in the effluent water of most LifeStraw models were either BMDL or well below the maximum recommended concentration (100ppb), some model replicates were near or above the guideline concentration. We recommend that the effluent silver concentrations of models L1 and L2 are closely monitored in further research.

- More than one time through the LifeStraw experiments mixed spiking of multiple test microbes was attempted. The ability to successfully perform assays and recover useable data from challenges with more than five test microbes was not possible despite months of preparatory research and a number of side experiments. It is thought that mixing a variety of microbes together in a laboratory setting results in

[1] An *n* of thirty is widely accepted as the minimum for good statistical analysis. An *n* of ten could be seen as appropriate if only considering mean lifetime LRV's.

low viability possibly due to physiochemical and/or morphological interactions between the test microbes (see Appendix B and C). Future microbiological evaluations should avoid mixed microbe challenges with more than five test microbes.

- Outside of improvements for a laboratory performance evaluation, the LifeStraw should be subject to a two-fold feasibility study. The first question that must be answered is whether or not the LifeStraw is a cost-effective, marketable good. A simple contingent valuation method such as a willingness to pay survey could provide significant insight into the consumer preferences in regards to the LifeStraw and other personal POU water purifiers. A second important question that should be answered when considering the widespread use of a water purification device is the extent to which the use of the purifier is correlated with a reduction of diarrheal disease. Likely the most important overarching goal of the LifeStraw is the reduction of diarrheal disease. It is recommended that a randomized control trial be done using the LifeStraw in a number of countries/settings that characterize its intended use.

APPENDIX A
7.1 E. coli experiments

Background

In the initial experiments for evaluation of the Lifestraw, *E. coli* strain KO11 was unstable in seeded test water when mixed in a "cocktail" with several other test microbes. Therefore, experiments were done troubleshoot this problem and come up with a solution to maintain *E. coli* stability and detectability in seeded test water.

A. Strain Type and Agar Experiment

Aims

- To compare two strains of *E. coli*: HMS174 (kanamycin-resistant) and KO11 (chloramphenicol-resistant)
- To compare recovery of each strain on tryptic soy agar (general purpose agar) with a more *E. coli*-selective agar (Bio-Rad Rapid *E. coli* II agar)
- To compare each of these agars with and without antibiotics

Methods

- Grow an overnight culture of each strain in the presence of antibiotics
- Grow a log-phase culture of each strain in the absence of antibiotics (TSB only)
- Make a ten-fold dilution series
- Membrane filter 9 ml volumes of selected dilutions for each strain
- Store log phase cultures at 4°C overnight

Results

Table A13: Concentrations (CFU/ml) of *E. coli* KO11 on 4 different agar media

KO11	CFU/ml
TSA	2.5E+08
TSA + chlor.	2.6E+08
Bio-Rad	2.3E+08
Bio-Rad + chlor.	2.30+08

BR: Bio-Rad
c.: chloramphenicol
Note: *E. coli* colonies on Bio-Rad plates were purple

Table A14: Concentrations (CFU/ml) of *E. coli* HMS174 on 4 different agar media

HMS174	CFU/ml
TSA	8.2E+07
TSA + kana.	8.6E+07
Bio-Rad	4.4E+07
Bio-Rad + kana.	3.9E+07

BR: Bio-Rad
k.: kanamycin
Note: *E. coli* colonies on Bio-Rad plates were blue and white

Results Summary:

- KO11 grows to a somewhat higher titer than HMS 174 during log phase.
- KO11 colonies were purple on Bio-Rad (as expected) while HMS 174 colonies were both blue and white, despite it being a pure culture. (Note: This may be due to differential expression of the LacZ gene by this strain of *E. coli*, which contains plasmids that may be lost or may have differential expression of the LacZ gene.)
- Bio-Rad gave slightly reduced detectability or recovery of HMS 174 cells as compared to TSA, but only by approximately ½ $\log_{10}$. This is not unexpected, as it is a differential-selective medium, which TSA is not (it is non-selective and non-differential.)
- Bio-Rad medium did not affect the recovery of KO11 cells as compared to TSA.

- The addition of antibiotics did not affect the recovery of KO11 cells on either agar medium type
- The addition of antibiotics did not affect the recovery of HMS 174 cells on TSA, but slightly decreased recovery on Bio-Rad agar medium

Conclusion

E. coli KO11 appears to be the better choice of strain type to use in our experiments based on its growth to a higher titer, consistently distinct colony morphology, comparable growth on selective medium (Bio-Rad) as compared to non-selective general medium (TSA), and comparable growth in the presence of antibiotics to growth in the absence of antibiotics.

B. *E. coli* Spiking Experiments

Aims

- To determine how well the selective agar (Bio-Rad Rapid *E. coli* II agar) with antibiotics grows log phase cultures (then stored overnight at 4°C) spiked into dechlorinated tap water as compared to standard TSA with antibiotics
- To determine how well membrane filtration works for these bacteria and agar media
- To determine how much mixing time is necessary for best bacterial detection and recovery

Methods

- Use broth culture with known log phase titer for each strain (determined in previous experiment) to determine volume to spike into 1 L of test water to give desired final concentrations
- Spike each strain into separate 1 L volumes of dechlorinated tap water
- Mix with stir bar
- At 5 minutes, make a ten-fold dilution series in PBS
- Membrane filter 10 ml volumes of selected dilutions in duplicate onto each of the following agars:
    - KO11: TSA with chloramphenicol and Bio-Rad with chloramphenicol
    - HMS174: TSA with kanamycin and Bio-Rad with kanamycin
- Repeat at 30, 60, and 120 minutes
- Incubate and count colonies to determine bacteria concentrations

Results

Table A15: Concentrations (CFU/ml) of *E. coli* HMS174 on 2 different agar

HMS174
Spike Conc: 3.3E+01

	TSA (CFU/ml)	Bio-Rad (CFU/ml)
5 min	3.6E+02	2.9E+02
30 min	3.6E+02	3.0E+02
60 min	3.4E+02	3.0E+02
120 min	3.6E+02	1.5E+02

Table A16: Concentrations (CFU/ml) of *E. coli* KO11 on 2 different agar media

KO11
Spike Conc: 3.0E+02

	TSA (CFU/ml)	Bio-Rad (CFU/ml)
5 min	9.3E+02	1.0E+03
30 min	9.5E+02	9.2E+02
60 min	1.1E+03	1.0E+03
120 min	9.9E+02	7.5E+02

Results Summary:

- Initial concentrations used to spike test waters (3.3E+01 for HMS174 and 3.0E+02 for KO11) were lower than the intended target because of a lab analyst error in computing the seed concentration correctly. Nevertheless, the seed concentration still allowed for effective detection of levels of *E. coli* and possible reductions in detection based on seed water conditions and plating methods.
- HMS174:
    - Concentrations of *E. coli* in seeded test water were similar for both TSA and Bio-Rad plates
    - Concentrations of *E. coli* recovered on both TSA and Bio-Rad media were ~1 $\log_{10}$ higher than expected
- KO11:
    - Concentrations of *E. coli* in seeded test water were similar for both TSA and Bio-Rad plates
    - Concentrations of *E. coli* recovered on both TSA and Bio-Rad were ~0.7 $\log_{10}$ higher than expected
- Recovery for either strain of *E. coli* did not appear to vary with stirring time

Conclusion

E. coli HMS174 and KO11 were similarly recovered, regardless of agar medium type or length of stirring in seeded test water.

APPENDIX B
8.1 Batch Experiments

Background

In the initial experiments on the evaluation of the Lifestraw, a number of microbes were unable to be recovered from spiked influent water in the desired concentrations, despite having known the concentrations and volumes spiked in to the same batch of test water. Therefore, batch experiments were done troubleshoot this problem and determine which microbes were most difficult to recover and to devise a plan to overcome this problem.

A. 5.11.07

Aims

- To replicate Lifestraw challenge water test conditions in an attempt to recover microbes at desired concentrations based on target seed concentrations in test water
- To determine the effect of mixing time on *E. coli* recovery and detection

Methods

- Two days prior to the experiment, grow an overnight culture of each bacterial strain, in the presence of antibiotics if necessary (*S. typhimurium* WG-45 and *E. coli* KO11)
- The day before the experiment, grow log phase cultures of each bacterial strain in the absence of antibiotics; titer log phase cultures
- Use known titers of log phase bacterial cultures, MS-2 coliphage (virus), *C. perfringens* spores, poliovirus, and *Cryptosporidium parvum* oocysts to spike into 25 L of test water to give desired final concentrations

- Spike each microbe into 25 L of dechlorinated tap water
- Mix with stir bar
- At 5 minutes:
    - Make a ten-fold dilution series in PBS (for bacterial assays)
    - Make another ten-fold dilution series in TSB (for MS-2 assay)
    - Remove an aliquot for poliovirus assay
    - Remove an aliquot for *C. parvum* assay
- For bacterial assays, membrane filter 10 ml volumes of selected dilutions in duplicate onto each of the following agars:
    - *S. typhimurium* WG-45: SS agar with nalidixic acid
    - *Enterococcus*: BEA (bile esculin azide agar)
    - *E. coli* KO11: Bio-Rad Rapid *E. coli* 2 agar with chloramphenicol
    - *C. jejuni*: blood agar with Preston's antibiotics solution; Campy Selective Blood-free agar
    - *C. perfringens*: TSC
    - *V. cholerae*: TCBS
- For MS-2 assay, perform SAL plaque assay on selected dilutions
- For poliovirus assay: cell culture assay for selected dilutions in quadruplicate using BGMK host cell line
- For *C. parvum* assay: filter 0.5 ml of undiluted sample through Nucleopore 13mm filters using the Quiagen filtration manifold, stain with fluorescent antibody reagent (Crypt-a-glo) and enumerate oocysts microscopically using an epifluorescent microscope

- Repeat above steps of microbial analysis of seeded test water at 30, 60, and 120 minutes
- Incubate and count bacterial colonies, virus plaques, or other assay units (i.e. number of *C. parvum* oocysts) to determine concentrations

Results

Table B17: Expected and detected concentrations (CFU/ml) of microbes

	Expected (CFU/ml)	5 min (CFU/ml)	30 min (CFU/ml)	60 min (CFU/ml)	120 min (CFU/ml)
***E. coli* KO11**	1.0E+04	ND	ND	ND	ND
***S. typhi.* WG-45**	1.0E+04	5.0E+00	2.3E+02	2.3E+02	5.0E+00
V. cholerae	1.2E+04	5.2E+02	9.E+02	3.4E+02	ND
Enterococcus	1.0E+04	3.2E+04	3.5E+04	4.3E+04	2.9E+04
***C. jejuni* (blood)**	1.2E+04	TNTC	TNTC	TNTC	TNTC
***C. jejuni* (bloodless)**	1.2E+04	8.8E+03	6.6E+03	5.4E+03	7.5E+03
MS-2	1.7E+04	6.4E+05	6.5E+05	4.5E+05	4.0E+05
***C. perfringens* spores**	1.6E+04	ND	ND	ND	ND
Polio virus	2.3E+04	2.6E+01	1.6E+03	1.2E+03	5.5E+02
***C. parvum* oocysts**	1.5E+03	1.3E+03	1.5E+03	1.2E+03	1.3E+03

ND: none detected
TNTC: too numerous to count

Results Summary:

- We were unable to recover *E. coli* KO11 at any time point
- At best, concentrations for *S. typhimurium* WG-45 were 2 $\log_{10}$ less than what was expected
- *V. cholerae* concentrations were 1 – 2 $\log_{10}$ less than what was expected; no data is available for the 120-minute time point
- *Enterococcus* was recovered at the concentrations expected
- *C. jejuni* concentrations were ~1 $\log_{10}$ less than what was expected when grown on *Campylobacter* Selective Blood-free agar; counts on Campy blood agar plates were

TNTC, as more than *C. jejuni* was able to grow on this medium than on the selective medium.

- MS-2 concentrations were 1 – 2 $\log_{10}$ higher than what was expected; it is likely that the previously determined concentration for our MS-2 stock was actually higher than we calculated or previously aggregated viruses in the stock became disaggregated.
- *C. perfringens* spores did not grow.
- Poliovirus concentrations were 1 – 3 $\log_{10}$ lower than what was expected; it appears that recovery increased with increased stirring time
- *Cryptosporidium parvum* oocysts were recovered as expected
- Except in the case of poliovirus, stirring time did not appear to have an effect on the recovery of the microorganisms

Conclusion

Enterococcus, MS-2, and *C. parvum* oocysts were recovered in approximately the concentrations expected. The remaining microbes (*S. typhimurium* WG-45, *V. cholerae*, *C. jejuni*, and poliovirus) were recovered in lower concentrations than expected. Loss of recovery varied from 1 – 3 $\log_{10}$ (or no recovery in the case of *E. coli* KO11) and in general recoveries did not appear to increase or decrease with stirring time (except in the case of poliovirus, where increased stirring time appears to have increased recovery).

B. 6.5.07 Experiment

Aims

- To replicate the 5/11/07 batch experiment which aimed to optimize challenge water conditions in an attempt to recover microbes at desired concentrations
- To increase the initial spike volumes (microbe concentrations) by ten-fold for bacteria to account for the reductions seen in the previous batch experiments in order to recover the overall microbe concentrations necessary for challenge studies

Methods

- Same as 5/11/07 experiment; however
    - Increase spike volumes by ten-fold for bacteria
    - Compare two methods for *C. perfringens* titering and analysis: membrane filtration and spread plating

Results

Table B18: Expected and detected concentrations (CFU/ml)

	Expected	Detected
***E. coli* KO11**	1.00E+05	3.53E+03
***S. typhimurium* WG-45**	3.16E+03	1.54E+03
V. cholerae	1.00E+04	4.07E+03
Enterococcus	3.16E+03	3.16E+03
C. jejuni	ND	0
MS-2	2.00E+04	1.45E+04
***C. perfringens* (spread plate)**	2.52E+05	2.88E+01
***C. perfringens* (membrane filtration)**	7.77E+06	4.14E+01
Polio	2.08E+04	ND
***C. parvum* oocysts**	5.01E+02	9.16E+01

ND: none detected
*: results pending

Results Summary:

- There was a 2 $\log_{10}$ decrease in *E. coli* KO11 concentrations

- *S. typhimurium* WG-45, *Enterococcus*, and MS-2 were detected in expected concentrations
- There was ~0.5 $\log_{10}$ decrease in *V. cholerae* concentrations; counts on duplicate plates varied considerably and colonies grew in clumps that were often difficult to read
- *C. jejuni* was not recovered on any agar medium plates
- There were considerable losses in concentrations for *C. perfringens* spores:
    - 4 $\log_{10}$ reduction using the spread plate method
    - 5 $\log_{10}$ reduction using the membrane filtration method
- There was a ~0.5 $\log_{10}$ reduction of *C. parvum* oocysts

Conclusion

E. coli KO11 concentrations are lower than expected. This could be due to a number of reasons, including susceptibility to antibiotics in the polio stock and/or the use of two different methods (i.e. spread plating for titering ("expected") and membrane filtration ("detected"). This needs to be further investigated. *S. typhimurium* WG-45, *Enterococcus*, and MS-2 were detected in expected concentrations. *V. cholerae* concentrations were 0.5 $\log_{10}$ lower than expected, however counts were slightly unreliable due to potential clumping of bacteria. *C. jejuni* was not recovered. Regardless of method used, there was considerable loss in *C. perfringens* spore concentrations. Our laboratory has noted similar issues with low spore recoveries in the past. Polio results will be reported when completed. There was ~0.5 $\log_{10}$ reduction in *Cryptosporidium parvum* oocysts

APPENDIX C
9.1 Individual vs. Mixed Batch Performance Study

Background

Due to the large number of challenge microbes, it was hypothesized that loss of detectability of certain microbes may have been an effect of microbial interactions. In attempt to investigate this possibility, experiments were conducted to assess recovery of microbes both individually and when mixed together in test water.

A. 5.17.07 Experiment

Aims

- To determine which microbes can be detected at target seeded concentrations individually in 2 L volumes of dechlorinated tap water
- To determine which microbes can be isolated or detected at expected levels when all other microbes are present (i.e. 1 L volumes from each individual volume were combined to create a 9 L volume with all microbes). That is, are there microbial interactions that hinder recoverability of some or all of the microbes?

Methods

- Two days prior to the experiment, grow an overnight culture of each bacterial strain, in the presence of antibiotics if necessary (*S. typhimurium* WG-45 and *E. coli* KO11)
- The day before the experiment, grow log phase cultures of each bacterial strain in the absence of antibiotics; titer log phase cultures

- Use known titers of log phase bacterial cultures, MS-2, *C. perfringens* spores, polio, and *Cryptosporidium parvum* oocysts to spike into 9 L to give desired final concentrations
- Spike each microbe into individual 2 L volumes of dechlorinated tap water; stir for 30 minutes
- Assay for each microbe as described above
- Mix 1 L water volumes of each seeded microbe from the individual 2-l test water volumes together to create a total of 9 L; stir for 30 minutes
- Assay for each microbe as described above
- Incubate and count bacterial colonies, virus plaques, or other assay units (i.e. number of *C. parvum* oocysts) to determine concentrations

Results

Table C19: Expected and Detected Concentrations (log_{10})

	Expected Ind. (log_{10})	Detected Ind. (log_{10})	log_{10} reduction E – D	Expected Mixed (log_{10})	Detected Mixed (log_{10})	log_{10} reduction E – D
E. coli	4.7	5.4	-0.7	4.0	4.4	-0.4
***S. typhi.*.* **WG-45**	4.7	3.8	0.9	4.0	3.1	0.9
V. cholerae	4.7	5.3	-0.6	4.0	3.7	0.3
Enterococcus	4.7	4.5	0.1	4.0	3.7	0.3
C. jejuni	ND	ND	ND	ND	ND	ND
C. perfringens	ND	0.0	ND	ND	-1.2	ND
MS-2	4.7	4.7	-0.0	4.0	3.7	0.3
poliovirus	5.0	3.3	1.8	4.4	2.5	1.8
Cryptosporidium	3.9	3.8	0.1	3.2	2.9	0.3

Ind.: Individual
E: expected
D: detected
ND: no data

Table C20: Detected Differences in Concentrations in Mixed (log_{10}) Seeded Test Waters
*By combining the 1 L volumes containing each microbe, we dilute each microbe 1:9. This table represents the log_{10} reduction beyond the expected dilution effect.

	log_{10} Reduction
E. coli	0.4
***Salm.* WG-45**	0.0
V. cholerae	1.0
Enterococcus	0.2
C. jejuni	ND
C. perfringens	ND
MS-2	0.3
Polio	0.1
Crypto	0.2

ND = no data

Results Summary:

- For all microbes (except poliovirus), there was a <1 log_{10} difference in what was expected and what was recovered, when analyzed both individually and when mixed together
- Poliovirus concentration was a ~1.8 log_{10} lower than what was expected when analyzed both individually and when mixed
- There was no growth of *C. jejuni* when analyzed individually or when mixed.
- *C. perfringens* spores did not grow for reasons that are now being investigated.
- For each microbe but one (*V. cholerae*), the log_{10} reduction beyond the expected dilution effect (1:9) ranged from 0.0 to 0.4.

Conclusion

Results indicate that recoverability of microbes, when assayed both individually and when mixed, is high (except in the case of poliovirus). Furthermore, the results suggest that while there is some loss of recoverability of microbes once mixed together, losses (beyond the

expected dilution effect) are generally low and likely due to random variation. *V. cholerae* is the only microbe whose concentration decreased by 1 $\log_{10}$ when mixed with other microbes. Overall, this experiment suggests that there are likely no interactions between microbes that affect recoverability when they are mixed together, if they are prepared individually in test water and then combined to create the test water mixture.

B. 5.23.07

Aims

- To determine which microbes can be isolated individually in 3.3 L volumes of dechlorinated tap water
- To determine which microbes can be detected and at what concentrations when all other microbes are present in seeded test water. Here, 2.8 L volumes of each individual seeded test microbe in separate volumes of test water were combined to create a composite 25 L volume with all test microbes). That is, are there microbial interactions that hinder recoverability of some or all of the microbes in a 25 L volume (the volume of seeded test water used for challenge assay)?

Methods

- Repeat as for 5.17.07, adjusting for the increase in final volume of test water to 25 L.

Results

Table C21: Expected and Detected Concentrations (log_{10})

	Expected Ind. (log_{10})	Detected Ind. (log_{10})	log_{10} reduction E – D	Expected Mixed (log_{10})	Detected Mixed (log_{10})	log_{10} reduction E – D
E. coli	4.9	4.1	0.8	4.0	3.0	1.0
***S. typhi.* WG-45**	4.4	3.5	0.9	3.5	2.5	1.0
V. cholerae	3.4	ND	ND	2.5	ND	ND
Enterococcus	4.9	4.4	0.5	4.0	3.7	0.3
C. jejuni	ND	ND	ND	ND	ND	ND
C. perfringens	ND	ND	ND	ND	ND	ND
MS-2	4.9	4.0	0.9	4.0	4.8	-0.8
Poliovirus	5.2	3.0	2.3	4.4	2.4	2.0
Crypto	4.0	ND	ND	3.1	3.0	0.1

Ind.: Individual
E: expected
D: detected
ND: no data

Table C22: Detected Differences in Concentrations Upon Mixing (log_{10})
*By combining the 2.8 L volumes containing each microbe, we dilute each microbe 2.8:25. This table represents the log_{10} reduction beyond the expected dilution effect.

	Log_{10} Reduction
E. coli	0.3
***Salm.* WG-45**	0.0
V. cholerae	ND
Enterococcus	-0.2
C. jejuni	ND
C. perfringens	ND
	-1.7
Polio	-0.3
Crypto	ND

ND: no data

Results Summary:

- For all microbes able to be detected, there was a </= 1 log_{10} difference in what was expected and what was recovered, when analyzed both individually and when mixed in the same volume of water.

- *V. cholerae* plates were incubated incorrectly due to a technical error, and therefore data were not available.
- There was no growth of *C. jejuni* when analyzed individually or when mixed
- *C. perfringens* spores did not grow for reasons that are now being investigated
- Poliovirus assays are being repeated due to cell culture problems that are being solved
- *C. parvum* individual concentration could not be determined due to a methodological error; this sample is being reanalyzed and results will be available soon
- For *S. typhimurium* WG-45, *Enterococcus*, and *E. coli* KO11, the $\log_{10}$ reduction beyond the expected dilution effect (2.8:25) ranged from -0.2 to 0.3.
- For MS-2, the $\log_{10}$ reduction beyond the expected dilution effect (2.8:25) was -1.7, indicating an increase in concentration; this may also be due to experimental variation or disaggregation.

Conclusion

This experiment provides useful information to troubleshoot methodological issues. The results do indicate recoverability of most test microbes is high, when assayed both individually and when mixed together. Loss of recoverability of *S. typhimurium* WG-45, *Enterococcus*, and *E. coli* KO11 once mixed with all other microbes was low, as was seen in the 5.17.07 experiment. However, this study found that MS-2 had larger variations in concentrations when mixed with all other microbes; this may be due to variability or disaggregation of virus aggregates in the virus stock. Due to technical error, there are no *V. cholerae* results available for this experiment. Overall, the data set from this experiment shows similar microbe detectability as did the 5.17.07 experiment. The results indicate that in

most cases, recoverability of most microbes in 25L of water is as is expected and recoveries do not appear to be affected by the presence of other microbes in the water volume.

REFERENCES

Abbott, S. and Janda M. (2006). *The Enterobacteria* (2nd ed.). Washington DC: ASM Press.

Ahn, J. (2006). *High dimension, low sample size data analysis.* PhD Dissertation. Thesis Call Number O.R. 2006 A2864. University of North Carolina at Chapel Hill, Chapel Hill.

Andrews, R. (2006). *Managing the Environment, Managing Ourselves*. New Haven: Yale University Press.

Ashbolt, N. Grabow, W and Snozzi, M. (2001). Chapter 13: Indicators of Microbial Water Quality. *Water quality - Guidelines, standards and health: Assessment of risk and risk management for water-related infectious disease* (pp. 28). Geneva: IWA Publishing.

Backer, H. and Hollowell, J. (2000). Use of Iodine for Water Disinfection: Iodine Toxicity and Maximum Recommended Dose. *Environmental Health Perspectives, 108*(8), 679-684.

Bayati, M. (1997). Even Carbons Have a Silver Lining. *Water Technology, 20*(7) 1-2.

Bitton, G. (2005). *Wastewater Microbiology* (3rd ed.). Hoboken, NJ. John Wiley & Sons, Inc.

Bonde, G. (1966). Bacteriological methods for estimation of water pollution. *Health Laboratory Science*(3), 124-128.

Brown, J. (2007). *EFFECTIVENESS OF CERAMIC FILTRATION FOR DRINKING WATER TREATMENT IN CAMBODIA.* PhD Dissertation. UNC, Chapel Hill.

Carincross, S. and Valdamis, V. (2006). Chapter 41: Water, sanitation and hygiene: simple, effective solutions save lives. In *Disease Control Priorities Project* (Second ed.): World Bank Publications.

CDC, (2005). *Safe Water System (SWS) Publications - PUR,* Date modified: August 24, 2005. http://www.cdc.gov/safewater/publications_pages/pubs_pur.htm. (Accessed November, 2007)

Clasen, T., Schmidt, W., Rabie, T., Roberts, I. & Cairncross, S. (2007). Interventions to improve water quality for preventing diarrhea: systematic review and meta-analysis. *British Medical Journal, 334* (7597), 782.

Clasen, T. & Menon, S. (2007). Microbiological performance of common water treatment devices for household use in India. *Int J Environ Health Res, 17*(2), 83-93.

Clasen, T. Nadakatti, S. and Menon, S. (2006). Microbiological performance of a water treatment unit designed for household use in developing countries. *Tropical Medicine and International Health, 2*(9)1, 5-9.

Clasen, T., Brown, J., Suntura, O. & Collin, S. (2004). Safe household water treatment and storage using ceramic drip filters: a randomized controlled trial in Bolivia. *Water Sci Technol, 50*(1), 111-115.

Clesceri, L. E., AD. Greenberg, AE. (Ed.). (1992). *Standard Methods for the Examination of Water and Wastewater* (18th ed.). Washington, DC: American Public Health Association.

Dufour, A. (1977). Escherichia coli: The Fecal Coliform. *Bacterial Indicators: Health Hazards Associated With Water*: American Society for Testing and Materials. Washington DC.

Edison, D and Miller, L. (2002). Iodinated resins: Separating myth from fact. *Water Technology, 25*(5) 1-3.

Eisenberg, J. N. Scott, J. C. & Porco, T. (2007). Integrating disease control strategies: balancing water sanitation and hygiene interventions to reduce diarrheal disease burden. *Am J Public Health, 97*(5), 846-852.

EPA. (1986). Ambient Water Quality Criteria for Bacteria, *EPA-440/5-84-002*. Washington D.C.: U.S. Environmental Protection Agency.

EPA. (2006). *National Primary Drinking Water Regulations*. EPA published manuscript: 40 CFR Part 142. Washington DC.

EPA. (1987). *Guidelines for Testing Microbiological Water Purifiers*. EPA published manuscript, Washington DC.

EPA. (2001). *Method 1602. Male-specific (F+) and somatic coliphage in water by single agar layer (SAL) procedure.* EPA published manuscript, Washington D.C.

Esrey, S., Feachem, R. & Hughes, J. (1985). Interventions for the control of diarrheal diseases among young children: improving water supplies and excreta disposal facilities. *Bull World Health Organ, 63*(4), 757-772.

Fewtrell, L. & Colford, J. Jr. (2005). Water, sanitation and hygiene in developing countries: interventions and diarrhoea--a review. *Water Sci Technol, 52*(8), 133-142.

Frandsen, V. LifeStraw Product Webpage (2007): studio NetSouls. www.lifestraw.com (Accessed October, 2007)

Frauchiger, D. (2007). Representative of Water Division at Vestergaard Frandsen. Personal communication. Chapel Hill.

Geldenhuys, J. and Pretorius P. (1989). The occurance of interic viruses in wolluted water, correlation to indicator organisms and factors influencing their numbers. *Water Sci Technol, 21*, 105-109.

Geldreich, E. E. (1969). Water pollution. Microbiology. *J Water Pollut Control Fed, 41*(6), 1053-1069.

Jensen, P., Ensink, J., Jayasinghe, G., van der Hoek, W., Cairncross, S., & Dalsgaard, A. (2003). Effect of chlorination of drinking-water on water quality and childhood diarrhoea in a village in Pakistan. *J Health Popul Nutr, 21*(1), 26-31.

Kosek, M., Bern, C. & Guerrant, R. (2003). The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull World Health Organ, 81*(3), 197-204.

Lantagne, D. Quick, R. and Mintz, E. (2007). HOUSEHOLD WATER TREATMENT AND SAFE: STORAGE OPTIONS IN DEVELOPING COUNTRIES: A REVIEW OF CURRENT IMPLEMENTATION PRACTICES. www.wilsoncenter.org/waterstories/Household_Water_Treatment.pdf (Accessed November 2008)

Leclerc, H., Edberg, S., Pierzo, V., & Delattre, J. M. (2000). Bacteriophages as indicators of enteric viruses and public health risk in groundwaters. *J Appl Microbiol, 88*(1), 5-21.

Mintz, E., Bartram, J., Lochery, P., & Wegelin, M. (2001). Not just a drop in the bucket: expanding access to point-of-use water treatment systems. *Am J Public Health, 91*(10), 1565-1570.

Moe, C. L., M.D. Sobsey, G.P. Samsa, and V. Mesolo. (1991). Bacterial indicators of risk of diarrhoeal disease from drinking water in the Philippines. *Bulletin of the World Health Organization, 69*(3), 305 - 317.

Murray et al. (2000) *Textbook of Respiratory Mechanics.* 3rd Edition. W.B. Saunders Company.

Nici L. et al. *Statement on Respiratory Muscle Testing*. (2001). The American Thoracic Society/European Respiratory Society.

NRC. (2004). *Indicators of Waterborne Pathogens*. Washington, DC: National Academies Press.

OECD and WHO. (2003). *Assessing Microbial Safety of Drinking Water*: London, UK. IWA Publishing.

Sahal, D. (1999). Removal of iodine by solid phase adsorption to charcoal following iodine oxidation of acetamidomethyl-protected peptide precursors to their disulfide bonded products: oxytocin and a Pre-S1 peptide of hepatitis B virus illustrate the method. *J Pept Res, 53*(1), 91-97.

Schlosser, O., Robert, C., Bourderioux, C., Rey, M., & de Roubin, M. (2001). Bacterial removal from inexpensive portable water treatment systems for travelers. *J Travel Med, 8*(1), 12-18.

Seelig, B. and Derickson, R. (1992). Activated Carbon Filtration, *Treatment Systems for Household Water Supplies*: North Dakota State University. www.ag.ndsu.edu/pubs/h2oqual/watsys/ae1029w.htm (Accessed November, 2007)

Sobsey, M. D. (2002). *Managing water in the home: accelerating health gains from improved water supply*. Geneva: WHO.

Sobsey, M. D. (November, 2007). Health impacts of silver. Personal Communication. Chapel Hill.

Souter, P. F. et al. (2003). Evaluation of a new water treatment for point-of-use household applications to remove microorganisms and arsenic from drinking water. *J Water Health, 1*(2), 73-84.

White, G, D. Bradley, and A. White. (1972). *Drawers of Water: Domestic Water Use in East Africa*. Chicago: University of Chicago Press.

WHO. (2001). *Safe Drinking Water*. Geneva, Switzerland. Publication for WHO/UNICEF Joint Monitoring Program.

WHO. (2007). *Combating waterborne disease at the household level* (PDF). ISBN 978 92 4 159522 3. IWA Publishing

WHO. (2006). *Guidelines for Drinking Water Quality*. (PDF) Geneva, Switzerland: World Health Organization. www.who.int/water_sanitation_health/dwq (Accessed November, 2007)