ORIGINAL RESEARCH

Assessment of the Efficacy of the First Water System for Emergency Hospital Use

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ABSTRACT

Objective: The First Water Responder B package water treatment device was evaluated for its ability to reduce the levels of spiked indicators and pathogens (*Escherichia coli,* MS2 coliphage, murine adenovirus, and *Cryptosporidium* oocysts) in a surface water to partially evaluate its appropriateness to be used to provide safe drinking water to hospitals during emergency situations.

Methods: Lake water was collected in 50-L carboys and spiked with selected indicators and pathogens (*E coli,* MS2 coliphage, murine adenovirus, and *Cryptosporidium* oocysts) at 2 different spike levels (low and high). This water was treated using the First Water Responder B, and the microorganisms were enumerated before and after treatment using US Environmental Protection Agency and Standard Methods. Microbial removal efficiencies were compared with Environmental Protection Agency guidelines.

Results: *E coli* spikes ranged from 2.9 to 1059 colony-forming units (CFU)/100 mL with removals to below detection limits (1 CFU/100 mL) to 2.8 CFU/100 mL or 0.98 to 3.5 $\log_{10}$ reductions. MS2 coliphage spikes ranged from 3 plaque-forming units (PFU) to 837 PFU/100 mL with removals to below detection limits (1 PFU/100 mL) to 11.7 PFU/100 mL or 0.65 to 1.9 $\log_{10}$ reductions. Murine adenovirus spikes ranged from 203 to 8410 most probable number (MPN) of infectious units/100 mL with removals to below detection limits (23 MPN infectious units/100 mL) to 1370 MPN infectious units/100 mL or 0.79 to $>$1.2 $\log_{10}$ reductions. *Cryptosporidium parvum* oocyst spikes ranged from 52 to 853 oocysts per liter with removals to below detection limits ($<$1 oocyst per liter) to 0.3 oocysts per liter or $>$2.2 to 3.4 $\log_{10}$ reductions.

Conclusions: Although the First Water system could remove a significant portion of the spiked organisms, it is recommended that this point-of-use system be coupled with chemical disinfection in a multiple-barrier approach to provide water of the highest reasonably achievable quality for hospital use in emergency situations.

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Key Words: drinking water at point-of-use, *E. coli*, MS2 coliphage, adenovirus, *Cryptosporidium parvum*

In 1854, John Snow, a London physician, and Reverend Henry Whitehead conducted an organized public health investigation and presented definitive proof linking the transmission of an infectious disease, cholera, with water supply. Since then, knowledge regarding waterborne disease has been fertile ground for research. Infectious diseases transmitted through contaminated water are categorized by biotype. Three major biotypes exist: bacteria, viruses, and parasites. Engineered treatment processes, applied commonly in developed nations, are designed to minimize the risks of waterborne transmission of infectious diseases.

The US Environmental Protection Agency (EPA) promotes a multiple-barrier approach (combining prevention, treatment, and disinfection) to ensure the safety of public water supplies. For example, under the Surface Water Treatment Rule, a public water supply may apply a combination of source water protection, filtration, and disinfection to meet regulatory requirements. Federal requirements nominally specify that treatment must achieve 99.99% (4 $\log_{10}$) virus, 99.9% (3 $\log_{10}$) *Giardia lamblia*, and 99% (2 $\log_{10}$) *Cryptosporidium* removal/inactivation.[1] For microbiological water purifiers, EPA specifies that systems should provide 99.9999 (6 $\log_{10}$) bacteria, 99.99% (4 $\log_{10}$) virus, and 99.9% (3 $\log_{10}$) *Giardia* cyst removal/inactivation.[2,3]

Within the hospital setting, control of both waterborne infectious agents and chemicals is critical to ensure the quality of care that is provided to patients. During emergency situations resulting from natural and human-made disasters, such as flooding or an explosion, safe water must be delivered to maintain patient care. This is especially true for patients deemed to be too sensitive to evacuate, although all of the patients in a hospital can be expected to be in a weakened state of health. Accordingly, hospital preparedness plans need to contain a contingency for providing short-term, alternative sources of clean water during situations in which reliable municipal water sources are not available. Such water sources would be used to maintain patient care until more reliable and acceptable alternatives can be mobilized.

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Options for providing clean, safe water for use in hospitals when municipal supplies are cut off include bottled water, maintenance of a backup well, and trucked-in water. Using bottled water may be limited to direct consumption and cooking. Additional supplies for bathing, laundry, or HVAC systems may be necessary. Water from a backup well or truck may need disinfection and/or treatment before it meets EPA criteria for safe drinking water.

A number of "package" water treatment devices have been developed and marketed for locations, such as the developing world, where potable water sources and piped municipal water are scarce. For hospital preparedness use, factors such as system mobility, ease of operation, need for ongoing maintenance, and advertised performance need to be considered. One system is a product called First Water. This system is promoted by the manufacturer for applications during emergency situations. (For additional information on the product, see *http://firstwaterinc.com*.) This research was designed to provide a scientific evaluation of the First Water product for removing/inactivating microorganisms from emergency water sources for the Wisconsin Hospital Preparedness Program. This study was not intended to determine the effective removal of all individual microorganisms, but was focused on the effective removal of selected indicators and pathogens.

METHODS

The study design involved treatment of natural water (from Lake Mendota, Madison, WI) spiked with representative bacterial and viral indicators (*Escherichia coli* and MS2 coliphage) and viral and protozoan pathogens (murine [mouse] adenovirus and C *parvum* oocysts). This lake water was used to be representative of a water source that may be available during a flood or other natural disaster. Natural water was selected over producing a synthetic laboratory water to contain a complex matrix of organic carbon, inorganic salts, turbidity, and background microorganisms. A volume of 50 L was used in each of 6 treatment tests. Nominally, 2 levels of spiked indicator and pathogen organisms were chosen based on levels found in highly protected source waters and anthropogenically influenced source waters.[4,5] The following sections summarize the samples and methods used in this treatment study.

Sample Preparation and Treatment

The treatment unit tested was a Responder B (System #1006-1013) consisting of a 5-µm wound filter, followed by a 0.5-µm nominal pore size carbon block filter and an ultraviolet (UV) unit rated for 10 000 hours of use. (A more detailed description of the product's components is available from First Water Systems/Aqua Sun, Marietta, GA.) The source water used in this study was collected on 5 occasions between October and November during turnover from Lake Mendota. The waters tested consisted of a 50-L sterile carboy of Lake Mendota water spiked with flow cytometer–sorted (*E coli* and C *parvum* oocysts) or culture-enumerated test organisms (MS2 coliphage and murine adenovirus). These organisms represent the 3 major pathogen biotypes. Water samples were collected and analyzed by enumerating each organism type (spiked and naturally occurring) before and after First Water treatment. To collect a treated sample, the hose orifices were flamed to remove any ambient microbial contamination, the spiked carboy was connected to the Responder B unit (contents constantly mixed), 10 L of treated water was wasted, and 20 to 30 L of treated water was collected in another sterile carboy. Subsamples for microbial enumeration after treatment were then collected from this second carboy. A total of 6 treatment tests were conducted.

Water Chemistry

The character of test water affects the removal and inactivation efficiency of any water treatment system. Because trucking raw water from a nearby surface water source for treatment and use may be necessary in some emergency situations, surface water of moderate water quality (Lake Mendota) was selected to evaluate the efficacy of the First Water system. Lake Mendota was chosen because of its physical proximity to our laboratory and its moderate water quality (some nutrients and algae). Lake samples were collected in 50-L carboys in October and November during lake turnover. To index the experiments so that results may be extrapolated to other water qualities based on literature information, a number of water-quality parameters were measured: pH, specific conductance, turbidity, hardness, and total organic carbon (TOC).

pH was measured using an Orion 520 Laboratory Instrument (Thermo, Beverly, MA). Specific conductance was measured using an Accumet Basic AB30 meter (Fisher Scientific, Pittsburgh, PA). Turbidity was measured using an HACH 2100N Turbidimeter (Loveland, CO). All of the meters were calibrated to appropriate standards on each day of use. Hardness was determined by summing the calcium and magnesium concentrations expressed as calcium carbonate according to Standard Methods 2340B.[6] The calcium and magnesium concentrations were measured using a Perkin-Elmer Optima Model 5300 inductively coupled plasma emission spectrometer (PerkinElmer, Waltham, MA) according to EPA Standard Method 200.7.[7] TOC was measured using Standard Methods 5310C-TOC by persulfate-UV oxidation.[6] All of the testing was conducted following the requirements of the National Laboratory Accreditation Program and the Wisconsin Environmental Laboratory Certification Program.[8]

Sample Spikes and Microbial Enumeration

The 3 biotypes for waterborne diseases include bacteria, viruses, and parasites. The organisms chosen for examination in this project, *E coli* (ATCC 25922), bacteriophage MS2 (ATCC 15597-B1), murine adenovirus (ATCC VR-550), and C *parvum* oocysts (Iowa strain, Sterling Parasitology, Tucson, AZ), represent bacteria, indicator virus, pathogenic virus, and parasites/protozoa, respectively. These organisms were chosen for their significance as indicators (*E coli* and bacteriophage MS2) or pathogens (adenovirus and C *parvum* oocysts) in drinking water.

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The project design incorporated 2 challenge levels for analysis: a low spike (eg, tens of organisms per 100 mL) and a high spike (eg, hundreds to thousands of organisms per 100 mL). These spike levels were chosen to represent variation that may be observed in a "source water of last resort." Monitoring of the highly protected source waters for the city of Boston enumerated fecal coliforms, for which *E coli* are a subset, on the order of 20 colony-forming units (CFU)/100 mL and F-specific coliphages (ie, viruses that infect bacteria), for which MS2 is the predominant type, on the order of 15 plaque-forming units (PFU)/100 mL.[4] In contrast, it has been demonstrated that storm flows can contain fecal coliforms higher than 1000 CFU/100 mL and protozoan pathogens (*Giardia* cysts) in excess of 200 cysts per 100 mL.[5] Although reliable quantitative assays for adenovirus in environmental waters are a topic of research, wastewaters have been demonstrated to contain on the order of 10 000 viral genetic copies per 100 mL.[9] It would be reasonable to expect significantly lower concentrations of adenovirus in source waters. In addition, research has demonstrated that drinking water treatment process effectiveness is affected by organism concentration; for example, Assavasilavasukul et al[10] reported that percent/log removals were greater when spiked organism concentrations were on the order of 1 million/L as compared with ambient concentrations of organisms (10s–100s/L). Therefore, the microorganisms in this study were chosen to cover the range not only of biotypes but also in susceptibility to treatments and organism concentration. Spike concentrations were also constrained by enumeration method sensitivity. Thus, the low spikes targeted 5 organisms per 100 mL for *E coli* and MS2, 500 most probable number (MPN) infective units/100 mL for murine adenovirus, and 50 oocysts per liter of *Cryptosporidium* oocysts, respectively. The high spikes targeted 1000 organisms per 100 mL for *E coli* and MS2, MPN infective units/100 mL for murine adenovirus, and 1000 oocysts per liter of *Cryptosporidium* oocysts, respectively. Actual spike levels were enumerated before treatment with the First Water system.

A high and a low spike were each tested in 3 separate tests. All of the organisms were spiked together to mimic a worst-case scenario. Appropriate duplicates and quality assurance controls (blanks, positive cultures, and negative cultures) were conducted for each analysis. An unspiked water sample was also processed as a control. Unspiked lake water, spiked lake water, and treated water were analyzed for each test. These subsamples were collected and tested within 6 hours of preparation.

E coli stocks were prepared by inoculating tryptic soy broth from frozen stock and incubated at 37° ± 1°C with shaking at 100 rpm. The resulting culture was flow counted on a BD FACSAria Cell Sorter (BD Biosciences, San Jose, CA) and frozen in 1-mL aliquots in 20% glycerol. Viable cell counts of these frozen stocks were confirmed using membrane filtration with incubation on modified m-TEC agar, EPA Method 1603[11] after several months of storage. Appropriate volumes of thawed, enumerated *E coli* stock were spiked into each carboy of test water. Enumerations for *E coli* from each subsample (unspiked lake water, spiked lake water, and treated water) from each test were conducted in triplicate using EPA Method 1603.

MS2 coliphage was prepared by enriching in tryptic soy broth containing *E coli* HS(pFamp)R (ATCC 700891) host and amended with streptomycin sulfate and ampicillin sodium salt (15 mg/L final concentration; Sigma Aldrich, St Louis, MO). Tween 80 (Sigma Aldrich) at a concentration of 0.2% vol/vol was added to maintain dispersal of the coliphage particles. The enrichments were filtered through a 0.45-µm syringe filter to remove host cells. The resulting suspension was enumerated using spot plates and the balance frozen in 20% glycerol/tryptic soy broth (vol/vol). Appropriate volumes of thawed, enumerated MS2 coliphage stock were spiked into each carboy of test water. Enumerations for MS2 coliphage from each subsample (unspiked lake water, spiked lake water, and treated water) from each test were conducted in duplicate using EPA Method 1602, the single agar layer method.[12]

Murine adenovirus was prepared by inoculating a BALB 3T3 (ATCC CCL-163) mouse cell line with murine adenovirus 1 and propagating for 2 weeks at 37° ± 1°C. The viruses were harvested and frozen in Dulbecco's modified Eagle's medium, 90%, and bovine calf serum, 10%. The murine adenovirus stock was enumerated using a 6- × 8-well most probable number (MPN) procedure. Briefly, a monolayer of BABL 3T3 cells was allowed to propagate in two 6- × 4-microwell plates for 3 days at 37° ± 1°C. Ten-fold serial dilutions of the murine adenovirus stock was produced using prewarmed Dulbecco's modified Eagle's medium, 98%, and bovine calf serum, 2%. One hundred microliters of each dilution were inoculated into 1 row of 6 wells. One row of 6 wells was uninoculated and served as a negative control. The MPN of the murine adenovirus stock was calculated using the following formula[6]:

$$\text{MPN/100 mL} = \frac{100 \times P}{(N \times T)^{1/2}}$$

where P = number of positive wells, N = volume of sample in all of the negative wells combined (milliliters), and T = total volume of sample in all of the wells of the relevant dilutions (milliliters).

C parvum oocyst spikes were prepared by flow sorting Iowa strain oocysts (Sterling Parasitology) on a BD FACSAria Cell Sorter into sterile centrifuge tubes containing reagent-grade water with 0.01% Tween 20 (Sigma Aldrich). Flow-sorted oocysts were stored at 4°C until use within 1 month of preparation. The samples (lake water, spiked lake water, and treated water) were analyzed using EPA Method 1623.[13]

RESULTS AND COMMENT

Lake water samples were collected on 5 different days between October and November and used for 6 different treatment tests. The water-quality analyses for the lake water used in this study are summarized in Table 1. Lake Mendota is slightly alkaline, with

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pH values ranging from 8.15 to 8.35. These are well within the Wisconsin NR 102 Water Quality Standards that state that surface waters shall be between pH 6.0 and 9.0.[14] The specific conductance of water is a measure of the ability of water to carry current and can provide a rapid estimate of the dissolved solids content of water. The values of the water used for testing range from 429 to 595 µS/cm. The state of Wisconsin has not established a surface water quality standard for specific conductivity. Streams in northeastern Wisconsin have been reported to have conductivity values between 300 and 1800 µS/cm.[15] The lake water used in this study was well within these typical values.

Turbidity is one measure of suspended matter in water. Relevant to the applications of this study, for drinking water systems with a filtration waiver (ie, disinfection only), turbidity before the first point of disinfectant application cannot exceed 5 nephelometric turbidity units (NTU).[16] With values ranging from 0.824 to 4.7 NTU, the water used in the present study lies within this turbidity range.

The hardness of the lake water used in this study ranged from 205 to 219 mg/L. This places Lake Mendota in the very hard category.[17] This is the case for areas of south and central Wisconsin; however, in northern Wisconsin, soils contain little limestone and surface waters contain much lower levels of hardness.

Drinking water TOC can range from <0.1 mg/L to 25 mg/L, whereas wastewater TOC can be >100 mg/L.[6] In drinking waters, organic matter serves as a precursor for disinfection byproducts and may enhance the survival of microorganisms.[18,19] Readily degradable organic matter can also lead to indicator regrowth.[20,21] The levels analyzed in the test waters, 5.7 to 6.3 mg/L, are within the typical ranges found in drinking water sources. Overall, the water quality of the lake water used in this study represents waters within the spectrum found in Wisconsin.

The results of the microbial enumerations from all 6 tests are summarized in Table 2. Each sample was enumerated in triplicate or duplicate and the mean values are presented in this table. These mean values were used to calculate the treatment efficiency in percentage of reduction and $\log_{10}$ removal.

E coli

The enumeration results for *E coli* are presented in Figure 1. This organism is 1 indicator organism system that has been reported to have specificity to fecal contamination along with

TABLE 1

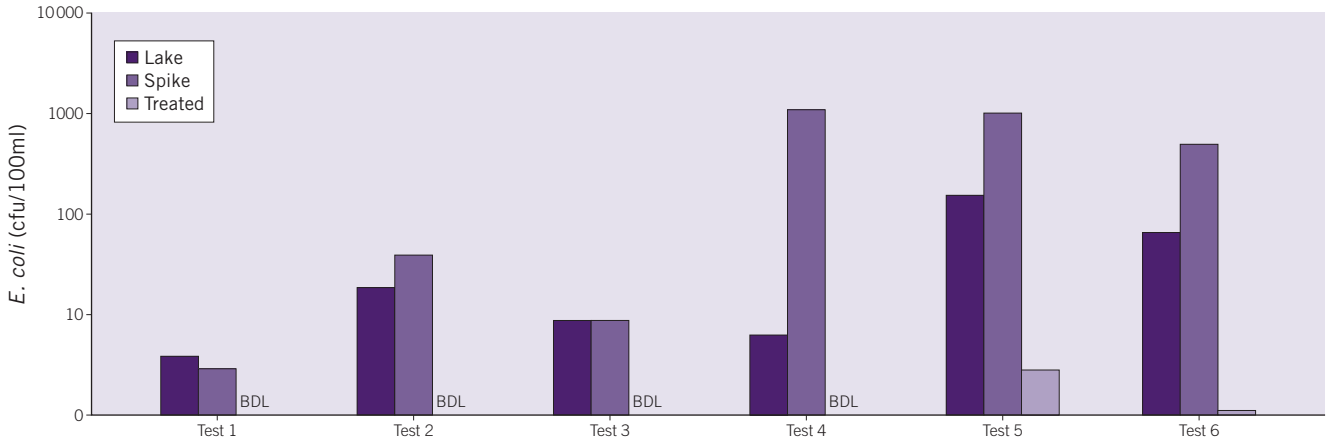
Summary of Lake Mendota Water Quality Used in Each Treatment Test

Parameter	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
pH	8.15	8.35	8.35	8.17	8.22	8.16
Specific conductance, µS/cm	595	429	429	460	460	429
Turbidity, NTU	0.824	3.64	3.64	2.35	4.7	4.5
Hardness, mg/L $CaCO_3$	200	207	207	205	216	219
TOC, mg/L	6.3	5.7	5.7	5.8	6.2	6.2

NTU=nephelometric turbidity units; TOC=total organic carbon.

FIGURE 1

Summary of *Escherichia coli* enumeration results



BDL=below detection limit.

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TABLE 2

Summary of Microbial Monitoring Data

Parameter	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Spiked water						
Escherichia coli, CFU/100 mL	2.9 (1.8)	38.9 (2.4)	8.7 (3.7)	1059 (72.9)	991 (159)	491 (75.4)
MS2 coliphage, PFU/100 mL	3 (1.4)	4 (0.7)	3 (2.2)	837 (152)	327 (66.6)	373 (30.6)
Adenovirus, MPN infective units/100 mL/100 mL	385 (NC)	203 (NC)	324 (17.7)	1630 (24.7)	7890 (18.2)	8410 (167)
Cryptosporidium, oocyst/L	52 (1.4)	61 (1.4)	64 (2.8)	811 (22.6)	788 (43.4)	853 (18.9)
Treated water						
E coli, CFU/100 mL	<1 (0)	<1 (0)	<1 (0)	<1 (0)	2.8 (2.2)	0.8 (1.2)
MS2 coliphage, PFU/100 mL	0.7 (0.6)	0.5 (0.7)	<1 (0)	11.7 (7.6)	9.3 (9.0)	9.7 (0.6)
Adenovirus MPN infective units/100 mL/100 mL	<23 (NC)	18 (NC)	31 (0.07)	106 (0.76)	1130 (15.9)	1370 (7.7)
Cryptosporidium, oocyst/L	<1 (0)	<1 (0)	<1 (0)	0.3 (0.5)	0.3 (0.5)	<1 (0)
Treatment efficiency						
E coli	>88% >0.98 log	>99% >2.1 log	>96% >1.4 log	>99.9% >3.5 log	99.7% 2.5 log	99.8% 2.8 log
Bacteriophage MS2	78% 0.65 log	89% 0.95 log	>94% >1.2 log	98.6% 1.9 log	97.1% 1.5 log	97.4% 1.6 log
Adenovirus	>94% >1.2 log	91% 1.0 log	86% 0.85 log	90.7% 1.0 log	85.7% 0.85 log	83.7% 0.79 log
Cryptosporidium	>99.4% >2.2 log	>99.5% >2.3 log	>99.5% >2.3 log	99.96% 3.4 log	99.96% 3.4 log	>99.96% >3.4 log

Standard deviations given in parentheses. CFU = colony-forming units; NC = not calculated (only 1 replicate measurement met quality assurance/quality control criteria); PFU = plaque-forming units.

coliphages and enterococci.[22,23] The inactivation and removal efficiency for *E coli* documented in this study ranged from >88% to >99.9%. For tests in which *E coli* densities were below detection limits (tests 1–4), the removal is based on the number of *E coli* microorganisms present in the spiked sample compared with the method detection limit (1 CFU/100 mL). *E coli* were detected in the treated sample in tests 5 and 6 with 99.7% (2.5 log) and 99.8% (2.8 log) removal, respectively. This demonstrates that treatment efficiency may decline over time, because the system had been operated for 5 previous runs totaling approximately 200 L of treated water. The inactivation and removal efficiencies for tests 1 through 4 may meet the EPA target of 6 $\log_{10}$ reductions; however, at the relatively low spike densities, this level of treatment efficiency could not be detected. Based on tests 5 and 6, additional treatment is needed to achieve the 6 $\log_{10}$ reduction goal for bacteria.

With the exception of *E coli* O157:H7 and other toxigenic *E coli*, environmental *E coli* are typically nonpathogenic indicators of water contamination by fecal or waste materials that could also contain pathogens. The regulations for public water supplies are based on total coliforms and *E coli*. A sample that is positive for total coliforms and *E coli* is considered unsafe for potable use under the Total Coliform Rule.[24] Again, based on tests 5 and 6, additional treatment is needed to reduce *E coli* to below detection limits (1 CFU/100 mL) for the treated water to be considered potable.

MS2 Coliphage

The enumeration results for MS2 coliphage are presented in Figure 2. Coliphages are enumerated as indicator organisms of fecal contamination.[25] As viruses, coliphages have similar fate and transport characteristics in the environment as pathogenic human viruses. Viruses are a significant organism to challenge filtration units because they are on the nanometer-size scale. Coliphage MS2 is 24 nm in diameter,[26] which is smaller than the nominal pore size of the carbon block filter. The Responder B unit removed coliphage MS2 from spiked lake water sample with 78% to 98.6% efficiency, with greater removal efficiencies demonstrated at higher spike concentrations. Active coliphage MS2 was recovered/detected in all of the treated samples except in test 3, in which MS2 was inactivated/removed to below assay detection limits (1 PFU/100 mL). These inactivation/removal efficiencies are below the EPA target of 99.99% for viruses.

MS2 coliphage is a member of the F-specific coliphage group. Coliphages, such as *E coli*, are indicator organisms. In this case, coliphages are used to signal the presence of fecal or waste contamination that may also contain pathogenic viruses. Coliphages were included as a potential indicator for viral pathogens for use in groundwater systems under the Ground Water Rule based on similarities in environmental survival.[27-29] If a groundwater supply tests positive for total coliforms, then retesting must be conducted, and coliphages are one acceptable target during retesting. The detection of any coliphage in a retest would be considered to be unsafe for potable use. Based on the results in this study, 5 of the 6 waters treated would not be considered suitable for potable use.

Murine Adenovirus

An adenovirus was chosen to challenge the First Water system for 2 primary reasons: adenoviruses (<100 nm) are smaller than the nominal pore size of the filter units; thus, it can be hypothesized that physical straining of these (and other) viruses by the filter will be a less important removal mechanism than it would be for larger microorganisms. Inclusion of adenovirus was also im-

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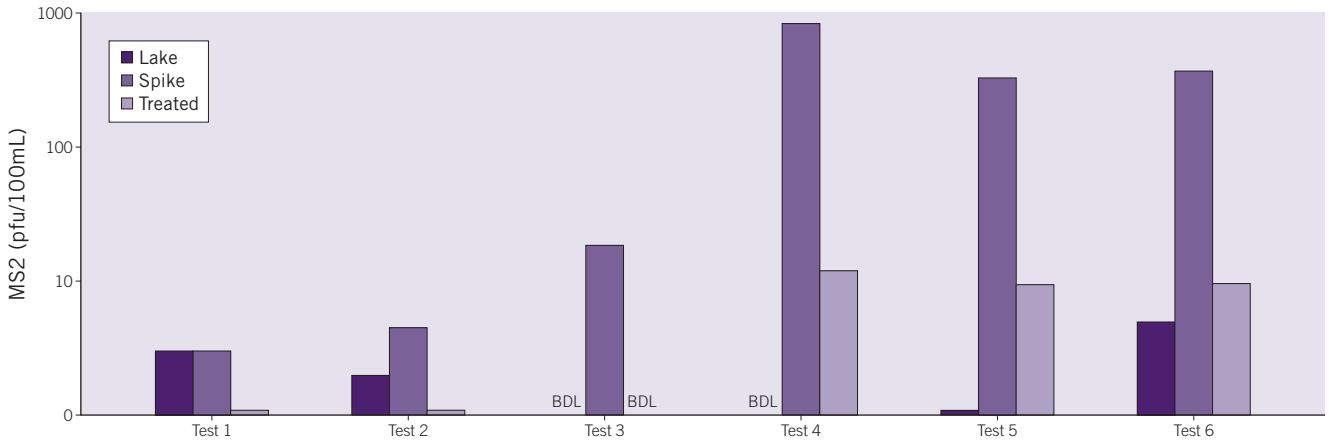
FIGURE 2

Summary of MS2 coliphage enumeration results



BDL=below detection limit.

portant because of its double-stranded DNA genome (rather than the single-stranded RNA genome typical for most other pathogenic viruses) and its associated higher resistance to UV inactivation.[30] Murine adenovirus was specifically selected for evaluation because it grows well in cell culture, allowing for changes in infectious numbers of virus to be determined and is safe (noninfectious to humans) to handle in the laboratory.

The enumeration results for murine adenovirus are presented in Figure 3. The inactivation and removal efficiency for adenovirus measured in this study ranged from 83.7% to 94%. Murine adenovirus was detected at concentrations above the assay detection limit in each of the 6 trials except test 1. These inactivation/removal efficiencies are below the EPA target of 99.99% for viruses.

Among pathogenic viruses that can be transmitted via the waterborne route, adenoviruses are unique in that they possess double-stranded DNA as their nucleic acid, whereas other known waterborne viruses contain RNA. Because of this, adenovirus has been demonstrated to be more resistant to UV treatment, one component of the First Water system, than other viruses.[31,32] Most assays for adenovirus in environmental waters are presence/absence rather than quantitative.[33] Risk assessment studies have reported that the occurrence of 1 infectious virus particle per 100 L results in an illness rate of about 8.3/1000.[34] Therefore, the results of 5 of the 6 waters treated would not be considered to be suitable for potable use with respect to adenovirus. It is suggested that additional treatment, such as chemical disinfection, be used in conjunction with First Water treatment during emergency situations.

C parvum

As the target organism of the EPA's Long Term 2 Enhanced Surface Water Treatment Rule, C *parvum* was included here as a benchmark organism. The enumeration results for *Cryptosporidium* oocysts are presented in Figure 4. The Responder B unit removed spiked oocysts to below assay detection limits (1 oocyst per liter in tests 1, 2, and 3 and 4 oocysts per liter in tests 4, 5, and 6) in 4 of the 6 trials. In the instances (tests 4 and 5) in which oocysts were enumerated in the treated water, 99.96% removal was achieved. Although Method 1623 is not a viability assay, the oocysts enumerated in the treated water were positive for intact nucleic acid by 4′,6-diamidino-2-phenylindole staining. This result suggests the presence of infectious oocysts. These inactivation/removal efficiencies meet the EPA target of 99.9% or 3 $\log_{10}$ reductions for *Cryptosporidium* oocysts.

The infectious dose of *Cryptosporidium* oocysts has been reported to range from between 30 and 132 oocysts; however, 1 report places the minimum infectious dose as low as 1 oocyst for individuals with compromised immune systems.[35] Under the Long Term 2 Enhanced Surface Water Treatment Rule, the EPA classifies utility source waters based on *Cryptosporidium* occurrence densities.[1] Treatment requirements vary with source water *Cryptosporidium* densities. The results from this study demonstrate that complete removal is achieved at the lower initial *Cryptosporidium* densities (52–64 oocysts per liter); however, low densities of oocysts are not removed when considering the higher spike (788–853 oocysts per liter). Considering that 1 oocyst may be a significant infectious dose for individuals with compromised immune systems, additional treatment may be necessary to ensure safe water during an emergency situation.

CONCLUSIONS AND RECOMMENDATIONS

The First Water system is marketed for applications in the developing world and natural disasters. The range of microorganism removals documented in this article (≥78%– 99.96% for all of the organisms in all of the the test runs) is similar to or better than the package water treatment systems tested or promoted for use in developing countries.[36-39] The infectious doses of microorganisms evaluated can be low in healthy individuals. Hospital pa-

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tients that are too sensitive to evacuate may be expected to be immunocompromised, or at a minimum of a lower health status. Therefore, they would be expected to demonstrate increased susceptibility to pathogenic microorganisms requiring a lower infectious dose to result in infection.[40]

The EPA promotes a multiple-barrier approach (combining prevention, treatment, and disinfection) for drinking water safety. Coupling chemical disinfection with application of a package filtration system such as First Water would be consistent with this approach. Therefore, it is recommended that chemical disinfection using chlorine or another inactivation process be applied in combination with a treatment similar to First Water for hospital preparedness applications. From the results of the present study, additional inactivation/removal for bacteria and viral microorganisms is needed to meet EPA's targets of 99.9999 (6 $\log_{10}$) bacteria and 99.99% (4 $\log_{10}$) virus removal/inactivation specified for microbial purifiers.

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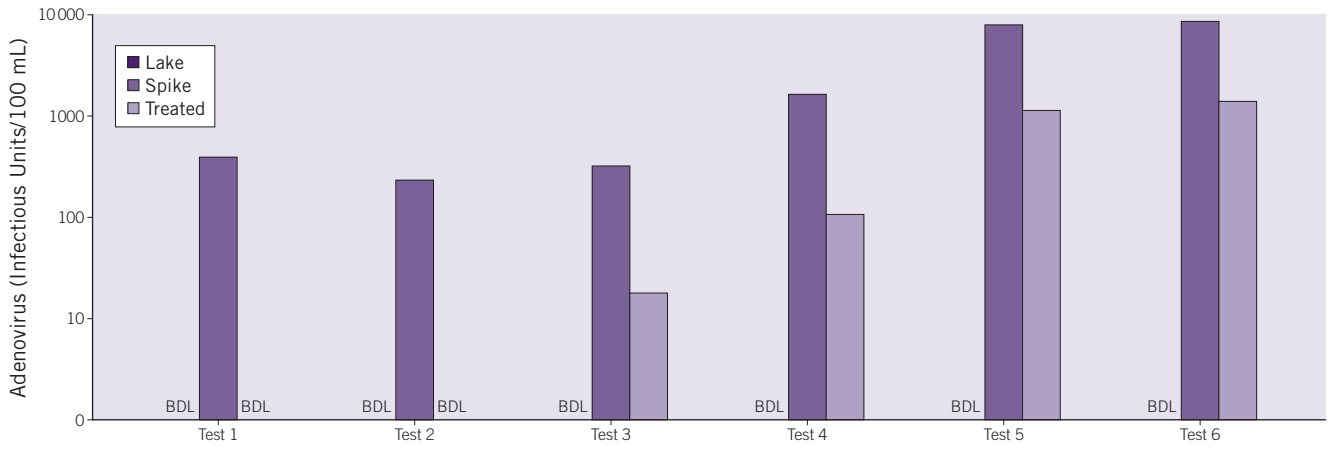
FIGURE 3

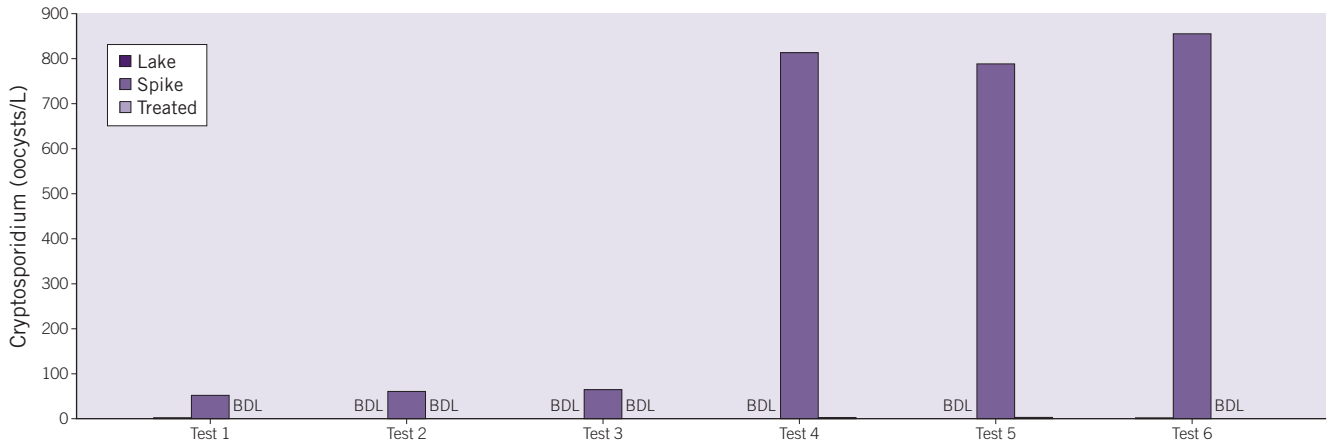
Summary of murine adenovirus enumeration results



BDL=below detection limit.

FIGURE 4

Summary of *Cryptosporidium* oocyst enumeration results



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