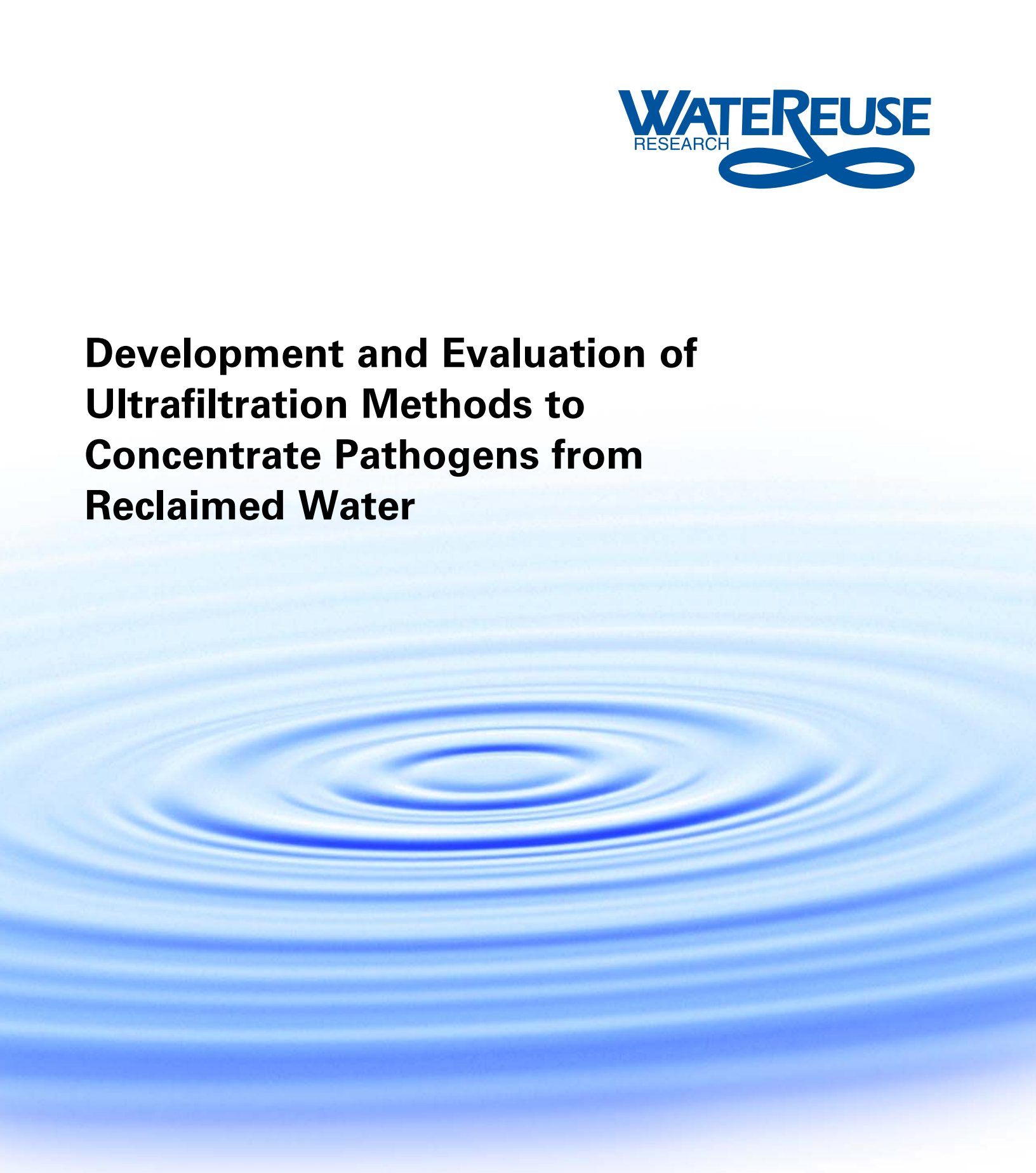
Development and Evaluation of Ultrafiltration Methods to Concentrate Pathogens from Reclaimed Water



WateReuse Research Foundation

Development and Evaluation of Ultrafiltration Methods to Concentrate Pathogens from Reclaimed Water

About the WateReuse Research Foundation

The mission of the WateReuse Research Foundation is to conduct and promote applied research on the reclamation, recycling, reuse, and desalination of water. The Foundation's research advances the science of water reuse and supports communities across the United States and abroad in their efforts to create new sources of high quality water through reclamation, recycling, reuse, and desalination while protecting public health and the environment.

The Foundation sponsors research on all aspects of water reuse, including emerging chemical contaminants, microbiological agents, treatment technologies, salinity management and desalination, public perception and acceptance, economics, and marketing. The Foundation's research informs the public of the safety of reclaimed water and provides water professionals with the tools and knowledge to meet their commitment of increasing reliability and quality.

The Foundation's funding partners include the Bureau of Reclamation, the California State Water Resources Control Board, the California Energy Commission, and the California Department of Water Resources. Funding is also provided by the Foundation's Subscribers, water and wastewater agencies, and other interested organizations.

Development and Evaluation of Ultrafiltration Methods to Concentrate Pathogens from Reclaimed Water

Christine L. Moe, PhD
Hubert Department of Global Health
Rollins School of Public Health
Emory University

Vincent Hill, PhD, PE
Parasitic Diseases Branch
National Center for Zoonotic, Vector-Borne and Enteric Diseases
Centers for Disease Control and Prevention

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Bureau of Reclamation
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Gwinnett County Department of Water Resources (GA)
Clayton County Water Authority (GA)
Centers for Disease Control and Prevention

WateReuse Research Foundation
Alexandria, VA

Disclaimer

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For more information, contact:
WateReuse Research Foundation
1199 North Fairfax Street, Suite 410
Alexandria, VA 22314
703-548-0880
703-548-5085 (fax)
www.WateReuse.org/Foundation

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CONTENTS

FIGURES

TABLES

ACRONYMS AND ABBREVIATIONS

ANOVA	analysis of variance
ASM	American Society for Microbiology
CCWA	Clayton County Water Authority
CDC	Centers for Disease Control and Prevention
CL	confidence limit
COV	coefficent of variation
Da	Dalton
FWH WRC	F. Wayne Hill Water Resource Center
HAV	hepatitis A virus
IFA	immunofluorescent antibody
IMS	immunomagnectic separation
MGD	million gallons of wastewater per day
MWCO	molecular weight cut-off
NTU	nephelometic turbidity unit
PBS	phosphate buffered saline
PEG	polyethylene glycol
SD	standard deviation
TOC	total organic carbon
UF	ultrafiltration
USEPA	United States Environmental Protection Agency

FOREWORD

The WateReuse Research Foundation, a nonprofit corporation, sponsors research that advances the science of water reclamation, recycling, reuse, and desalination. The Foundation funds projects that meet the water reuse and desalination research needs of water and wastewater agencies and the public. The goal of the Foundation's research is to ensure that water reuse and desalination projects provide high-quality water, protect public health, and improve the environment.

An Operating Plan guides the Foundation's research program. Under the plan, a research agenda of high-priority topics is maintained. The agenda is developed in cooperation with the water reuse and desalination communities including water professionals, academics, and Foundation Subscribers. The Foundation's research focuses on a broad range of water reuse research topics including:

- Defining and addressing emerging contaminants;
- Public perceptions of the benefits and risks of water reuse;
- Management practices related to indirect potable reuse;
- Groundwater recharge and aquifer storage and recovery;
- Evaluation and methods for managing salinity and desalination; and
- Economics and marketing of water reuse.

The Operating Plan outlines the role of the Foundation's Research Advisory Committee (RAC), Project Advisory Committees (PACs), and Foundation staff. The RAC sets priorities, recommends projects for funding, and provides advice and recommendations on the Foundation's research agenda and other related efforts. PACs are convened for each project and provide technical review and oversight. The Foundation's RAC and PACs consist of experts in their fields and provide the Foundation with an independent review, which ensures the credibility of the Foundation's research results. The Foundation's Project Managers facilitate the efforts of the RAC and PACs and provide overall management of projects.

The Foundation's primary funding partners include the Bureau of Reclamation, California State Water Resources Control Board, the California Energy Commission, Foundation Subscribers, water and wastewater agencies, and other interested organizations. The Foundation leverages its financial and intellectual capital through these partnerships and funding relationships.

The overall goal of this study was to develop an ultrafiltration method that would reliably provide 50% or greater recovery of the test microorganisms (bacteriophages MS2 and Phi X174, *E. coli*, *C. perfringens* spores, and *C. parvum* oocysts) from reclaimed water. The research team conducted a literature review and performed laboratory experiments to achieve the goal.

David L. Moore
Chair
WateReuse Research Foundation

G. Wade Miller
Executive Director
WateReuse Research Foundation

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This project was funded by the WateReuse Research Foundation in cooperation with the Centers for Disease Control and Prevention, the F. Wayne Hill Water Reclamation facility in Gwinnett County, Georgia, and the Shoal Creek Water Reclamation Facility in Clayton County, Georgia.

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Principal Investigators

Christine L. Moe, PhD
Center for Global Safe Water at Emory University
Hubert Department of Global Health
Rollins School of Public Health
Emory University

Vincent Hill, PhD, PE
Parasitic Diseases Branch
National Center for Zoonotic, Vector-Borne and Enteric Diseases
Centers for Disease Control and Prevention

Research Project Team

Pengbo Liu, PhD, *Center for Global Safe Water, Emory University*
Trisha Johnson, *Centers for Disease Control and Prevention* and *Atlanta Research and Education Foundation*
Donghyun Hahn, *Centers for Disease Control and Prevention* and *Atlanta Research and Education Foundation*
Jothikumar Narayanan, PhD, *Centers for Disease Control and Prevention*

Project Advisory Committee

Michelle Chapman, *Bureau of Reclamation*
Ricardo DeLeon, *Metropolitan Water District of Southern California*
April Garcia, *Sanitation Districts of Los Angeles County*
Rich Mills, *California State Water Resources Control Board*

EXECUTIVE SUMMARY

After a review of literature on the ultrafiltration (UF) and our direct experience with UF procedures, this project was designed with the goal of developing a simple, effective UF procedure to detect multiple pathogens from reclaimed water. When the experimental data indicated that calf serum blocking of ultrafilters did not result in substantially higher microbial recoveries from reclaimed water, the project focused on elution as the key technique for achieving high recoveries. The results from 10-L and 100-L experiments with reclaimed water from two different facilities indicate that UF, followed by a simple elution technique using 0.01% Tween 80, can result in effective simultaneous recovery of diverse microbes, including vegetative bacteria, spore-forming bacteria, viruses, and parasites, from reclaimed water. The performance goal set for this project (at least 50% recovery for all study microbes at a 95% confidence limit) was largely met, with only a few exceptions. Because of the inherent variability associated with these types of water matrices, sample processing, and microbial assays, it is not likely that additional sample processing or use of different reagents/techniques would result in significantly higher recoveries or less variability. It has been demonstrated previously that properly manufactured ultrafilters do not allow significant microbial breakthrough (Hill et al., 2005; Olszewski et al., 2005). Thus, the microbial losses indicated by the recovery data in this study are likely due to adsorption of the microbes to the filter media or physical entrapment within the filter media. However, the observed losses of *E. coli* during the 100-L high-seed experiments suggest that there was also die-off of these laboratory-grown *E. coli*.

In addition to demonstrating good microbial recovery and associated sensitive detection limits, we also determined that none of the three ultrafilters studied provided significantly better performance. This useful finding suggests that the UF-elution method will work equally well with a variety of ultrafilters and that staff at water reclamation facilities can select the most appropriate ultrafilter based on cost or operational characteristics. Performance estimates for the UF-elution method with ultrafilter cartridges that were not included in this project should be made based on test results using these alternative cartridges. A summary of ultrafilter specifications and operational characteristics for this study is provided in Table 3.1. As anticipated, the ultrafilter with the largest pore size and greatest surface area (the Baxter Exeltra Plus 210) was associated with higher filtration rates and lower pressures to achieve these rates. However, these differences in operational conditions are minor because of the similar microbial recovery performances determined for each ultrafilter type.

CHAPTER 1

STUDY OBJECTIVES

1.1 OBJECTIVES OF STUDY

- Perform a literature review on the use of ultrafiltration for concentration and sampling various water types;
- Perform laboratory experiments investigating microbe recovery under different ultrafiltration conditions using three different types of ultrafilters;
- Perform laboratory experiments investigating ultrafiltration method performance for treated wastewater from two different water reclamation plants;
- Perform laboratory experiments investigating the performance of the ultrafilter method for detecting low levels of enteric microbes.

The overall goal was to develop an ultrafiltration method that would reliably provide 50% or greater recovery of the test microorganisms (bacteriophages MS2 and Phi X174, *E. coli*, *C. perfringens* spores, and *C. parvum* oocysts) from reclaimed water. The experiments to achieve this goal are described in Chapter 3 of this report. The literature review is summarized in Chapter 2.

CHAPTER 2

REVIEW OF THE LITERATURE

Before starting our experimental work, we searched PubMed and ScienceDirect and identified 18 relevant publications on ultrafiltration methods from 1974 to 2005. We carefully reviewed these research papers and summarized the key information in a matrix, including: publication journal, year of publication, author, type of water, volume of water, filter type, membrane type, membrane size, conditions for filter blocking, elution and backwash, test microorganism, and recovery rate. This literature review is available on the website of the Center for Global Safe Water at Emory University (http://www.sph.emory.edu/CGSW/index.php)

Previous research on ultrafiltration for microbial concentration from water has examined a variety of filters made from polyacrylonitrile, polysulfonate, polyethersulfone, and cellulose acetate. The molecular weight cutoff size of these filters ranged from 6000 to 100,000 Daltons. The waters used in these evaluations were either laboratory waters (sterile, distilled, or deionized water), tap water or environmental waters (groundwater, surface water). One previous study by Feng et al. (2003) included the examination of reclaimed water. The test microbes used in these studies included various bacteriophage, human viruses (poliovirus and hepatitis A virus), vegetative bacteria (*Salmonella*, *E. coli*), Bacillus spores, and protozoan oocysts (*Cryptosporidium, Giardia*). Various ultrafiltration approaches, such as filter blocking, elution, and backwash were used in these studies. A large range of microbial recovery rates are reported (0.4–110%); however, this is not surprising given the diversity of test conditions, waters, and microbes.

CHAPTER 3

EXPERIMENTAL EVALUATION OF ULTRAFILTRATION FILTERS AND SELECTED TEST PARAMETERS

3.1 EXPERIMENTAL DESIGN

In June 2006, the Centers for Disease Control and Prevention (CDC) and Emory University staff started working on the ultrafiltration procedure, as well as preparing and testing the protocols for the microbiological assays used for each of the test microorganisms in the seeding experiments. Assay preparation included preparing microbial seed stocks, verifying membrane filtration methods to enumerate *E. coli* and *C. perfringens*, and verifying plaque assays to enumerate bacteriophage MS2 and Phi X174. A series of ultrafiltration experiments were planned to evaluate the effectiveness of the following four parameters for recovering viruses, bacteria, and parasites seeded into 10- and 100-L samples of reclaimed water:

- Filter type
- Filter blocking with calf serum
- Surfactant elution
- Backflushing of ultrafilters

Samples of tertiary-treated wastewater were collected from the F. Wayne Hill Water Reclamation facility in Gwinnett County, Georgia and the E. L. Huie Constructed Wetlands facility associated with the Clayton County (Georgia) Shoal Creek Water Reclamation Facility.

The study variables and operating parameters were selected based on the literature review and results of previous ultrafiltration research (Hill et al., 2005; Hill et al., 2007; Morales-Morales, et al., 2003; Olszewski et al., 2005; Polaczyk et al., 2008;). These research studies identified the importance of various ultrafiltration parameters, including filter pretreatment (i.e., blocking) and microbe desorption (e.g., by elution or backflushing). Hill et al., 2007 showed that an ultrafiltration procedure could be effective for concentrating 100-L tap water samples at a filtrate rate of ~1.2 L/min and corresponding cross-flow rate of ~1.7 L/min and pressure of ~13 psi. These flow rate and pressure conditions were selected as the baseline conditions for Project WRF-04-013. Experiments were planned to investigate alternative flow rates and system pressures if the final ultrafiltration procedure did not effectively meet the method performance goal for the project.

A method performance goal of achieving 50% or greater recovery for all study microbes was set for this project based on the formula:

$$\text{Avg. \%} - 2 \times \text{s.d.} \geq 50\%$$

This was an ambitious performance standard to use as a goal, and the feasibility of this goal could not be ascertained without experimental data specific to this project. In general, sampling techniques are considered to be effective if they result in method recovery efficiencies of 50% or greater, on average (i.e., without respect to performance variability).

For example, the acceptance criteria for U.S. Evironmental Protection Agency's (USEPA's) Method 1623 were established to be 61% precision, with a mean recovery of 13–111% for *Cryptosporidium* recovery in 10-L water samples (USEPA, 2001).

For replicate experiments, we calculated the mean percent recovery and the standard deviation for each target microbe. The 95% confidence limits (CLs) were calculated as the mean recovery $\pm$ 1.96 x standard error of the mean recovery. The coefficient of variation (COV) was calculated as the standard deviation divided by the mean recovery. Statistical analyses of the experimental results included two sample t-tests to compare the recovery rates between two methods (such as blocking vs. no blocking, with or without elution, etc.) and analysis of variance (ANOVA) to compare methods and/or filter type by microbe.

In May and June 2006, we collected wastewater samples from various locations at the F. Wayne Hill Water Reclamation Plant to determine an appropriate location for sample collection for this project. The samples from the F. Wayne Hill plant were obtained at the following locations in the facility: secondary effluent, ultrafiltration process effluent, granular media filter process effluent, and final plant effluent. Forty-liter samples were collected at each location. A portion of these samples were processed by ultrafiltration for quantitative analysis of bacteriophages, *Cryptosporidium parvum* oocysts, and *Giardia intestinalis* cysts. Other parameters tested were: fecal coliforms, *E. coli*, *Bacillus spp.*, *Clostridium perfringens*, and noroviruses. The concentrations of certain microbes measured in the secondary effluent and granular media effluent were sufficiently high to make both of these locations unsuitable for collecting wastewater samples for the ultrafiltration method development experiments. However, analyses of the ultrafiltration process effluent indicated that this location had very low microbial concentrations and thus was a good source of reclaimed water for the planned experimental work.

3.2 TEST FILTERS AND ULTRAFILTRATION SET-UP

One of the project goals was a better understanding of whether different commercially available, disposable, hollow-fiber ultrafilters provide different levels of microbial recovery performance when applied to reclaimed water. Commercially available, disposable hollow-fiber ultrafilters are sold by healthcare companies, including Fresenius Medical Care (Fresenius), Baxter, and Minntech, because they are used for kidney dialysis. Reusable hollow-fiber ultrafilters are also available commercially, but these filters tend to be more expensive and require considerable re-processing effort to disinfect and clean them for reuse. Prior to the WateReuse Research Foundation project with Emory University, the CDC had considerable experience and success with hollow-fiber ultrafilters from Fresenius (Lexington, MA). The Fresenius ultrafilters are comprised of polysulfone filter fibers that have an approximate pore size (also referred to as molecular weight cut-off or MWCO) of 30,000 Daltons (Da). The F200NR disposable ultrafilter has a total filter area of 2.0 m^2 and a fiber inner diameter of 200 µm. Because pore size has the potential to affect filtration efficacy, we decided to also examine the HPH 1400 "hemoconcentrator" ultrafilters sold by Minntech (Minneapolis, MN). These filters are also comprised of polysulfone hollow fibers but have a reported pore size of 65,000 Da (i.e., larger than the F200NR) and a total filter area of 1.3 m^2. A third hollow fiber ultrafilter examined in this study was the Exeltra Plus 210 filter sold by Baxter (Deerfield, IL). This ultrafilter has the same approximate pore size (70,000 Da) as the HPH 1400, but is comprised of hollow fibers made of cellulose triacetate and has a total filter area of 2.1 m^2. The three hollow fiber ultrafilters evaluated in this project are shown in Figure 3.1. The operational conditions and specifications for the three filters are indicated in Table 3.1. Figure 3.2 shows the set-up of 10-L ultrafiltration experiments.

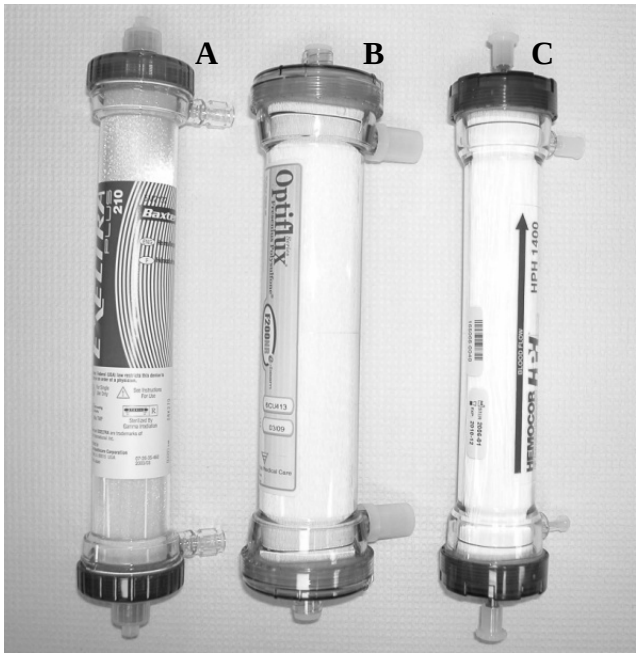

Figure 3.1. Ultrafiltration cartridges studied in this project: Baxter Exeltra Plus 210 (A), Fresenius Optiflux F200NR (B), and Minntech Hemocor HPH 1400 (C).

Table 3.1. Operational Conditions and Specifications for Three Hollow-Fiber Ultrafilter Types

Operational Condition	Fresenius F200NR	Minntech HPH 1400	Baxter Exeltra Plus 210
Filtrate rate (mL/min)	1200 ± 150	1200 ± 100	1300 ± 86
Pressure (psig)	13 ± 1.8	12 ± 2.5	10 ± 1.9
MWCO (Daltons)	~ 30,000	65,000	70,000
Filter area (m^2)	2.0	1.3	2.1
Hollow-fiber media	Polysulfone	Polysulfone	Cellulose triacetate

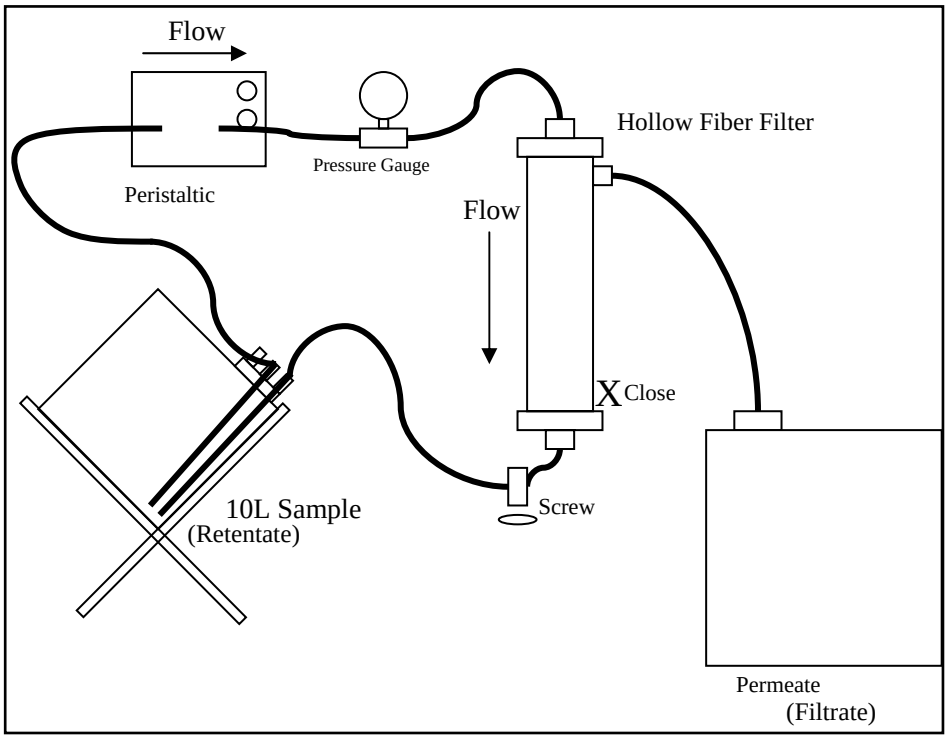

Figure 3.2. Schematic of experimental set-up for 10-L ultrafiltration experiments as described by Hill et al. (2005).

3.3 WATER RECLAMATION PLANTS AND RECLAIMED WATER CHARACTERISTICS

Because of rapidly growing population and limited water supplies, two water utilities in the metropolitan Atlanta area are currently practicing indirect potable water reuse or have a permit to practice indirect potable water reuse. The Gwinnett County Public Utilities in Gwinnett County (population approx. 700,000) operates the F. Wayne Hill Water Resources Center (FWH WRC) that currently treats 20 million gallons of wastewater per day (MGD) and is expanding to 60 MGD. The treatment train in this facility currently consists of: enhanced biological treatment and advanced tertiary treatment (chemical coagulation, recarbonation clarifiers, granular media filtration, preozonation, granular activated carbon, and post-ozonation; see Figure 3.3). The 2005 expansion includes the addition of Zenon's ZeeWeed membrane ultrafiltration. The FWH WRC has received approval from the Georgia Environmental Protection Division to discharge 40 MGD into Lake Lanier—a manmade lake that covers 38,000 acres and serves as both a drinking water source and an important recreational water body. The current Phase II Permit Limits are: COD – 25 mg/l; NH3 – 0.5 mg/l; Phosphorus – 0.13 mg/l, Turbidity – 1 NTU, fecal coliforms – 23 CFU/100 ml. For two years, the FWH WRC has produced effluent with <0.2 NTU turbidity, <30 ppb Phosphorus, and <1 fecal coliform/100 ml.

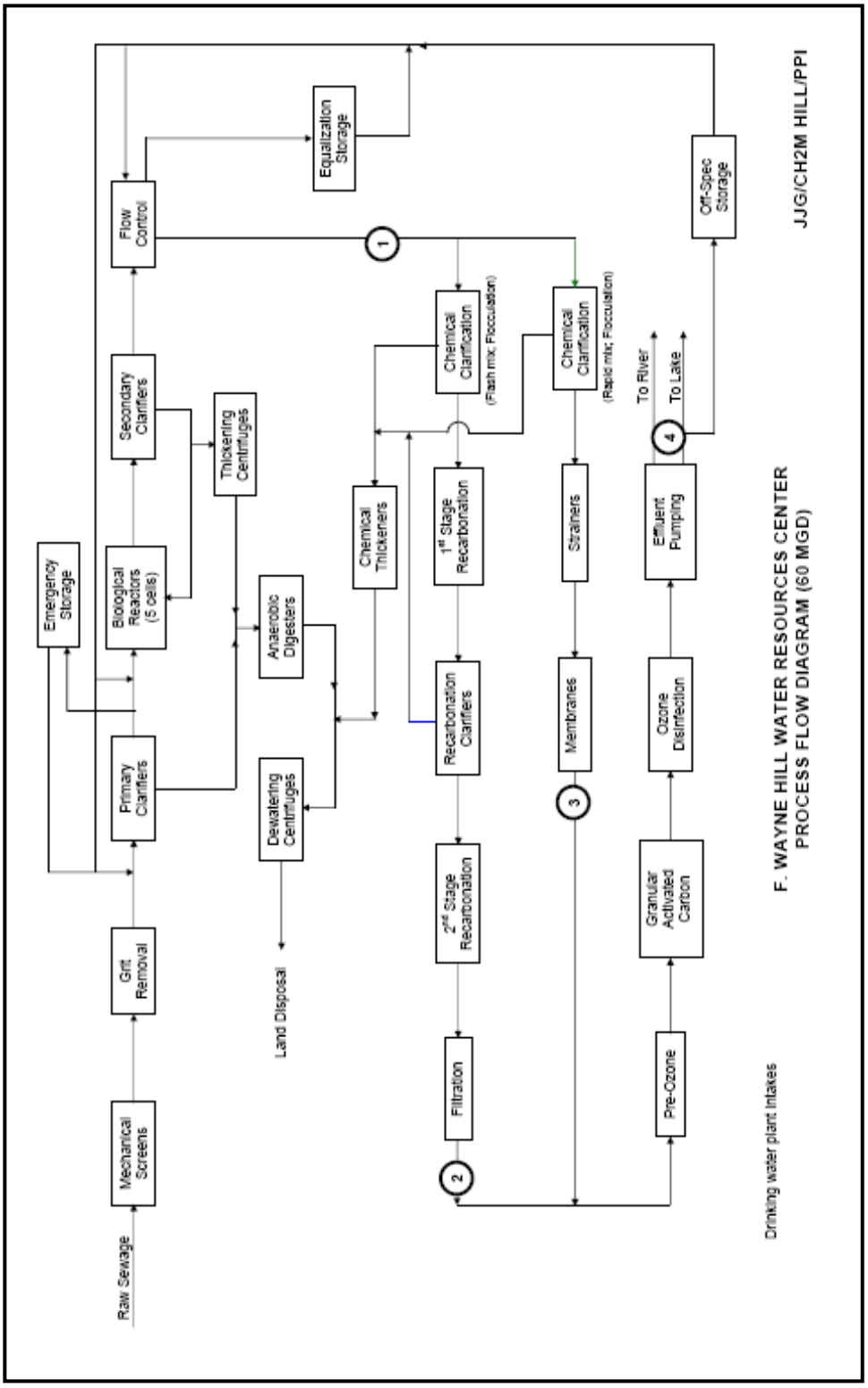

Figure 3.3. Diagram of F. Wayne Hill Water Reclamation Facility in Gwinnett County, GA. Sampling point is indicated at Point 3.

Clayton County, Georgia is one of the fastest growing areas in the United States with a 21% increase in population from 1980 to 1990 and a 15% increase from 1990 to 1998. However, Clayton County has very limited water resources. The Clayton County Water Authority (CCWA) currently operates three water production plants and provides water for approximately 260,000 people. Since the 1970s, the CCWA has been a leader in land application of treated wastewater and developed a spray irrigation system that covered 2400 acres with 20,000 sprinkler heads and more than 300 miles of pipe. However, because of the high maintenance costs, aging infrastructure, and the need to increase their capacity to reclaim wastewater, the CCWA has recently moved to a constructed wetlands approach for sustainable wastewater management and reservoir recharge.

The W.B. Casey Water Reclamation Facility is a 24 MGD plant that provides advanced secondary treatment. The final effluent from this plant is pumped to the E. L. Huie Constructed Wetlands. The average residence time in the constructed wetlands is five to seven days, followed by discharge to the Shamrock Reservoir and Blalock Reservoir (see Figure 3.4).

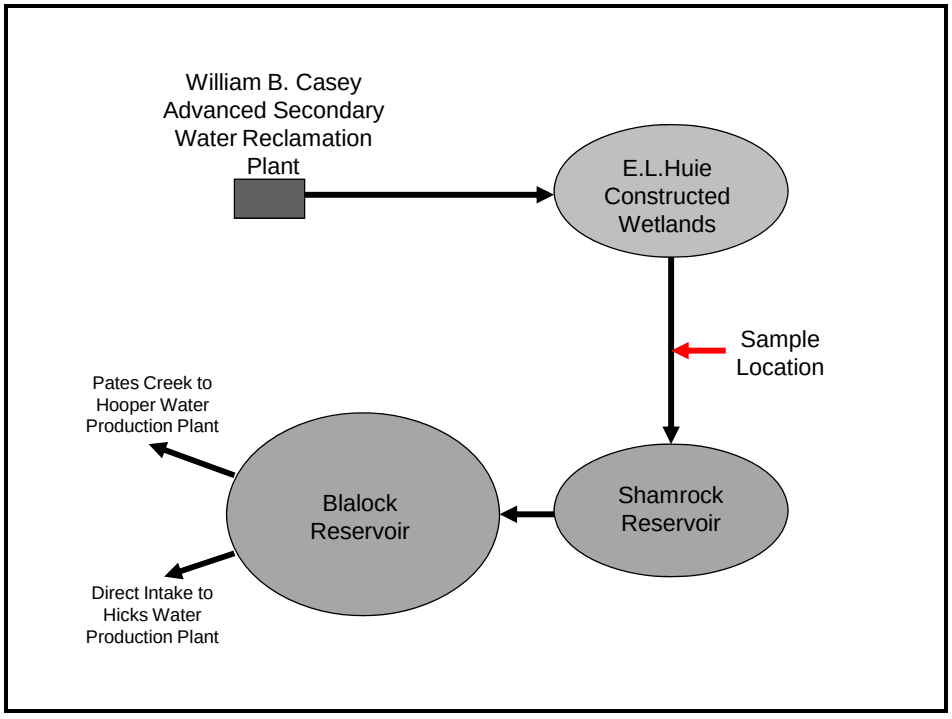

Figure 3.4. Diagram of constructed wetlands water reclamation system in Clayton County, GA

Reclaimed water collected from the F. Wayne Hill and E. L. Huie facilities was tested for an array of water quality parameters (see Table 3.2). Specific conductance, pH, and total organic carbon (TOC) were similar between the two sources of reclaimed water. Only turbidity differed significantly between the two sites. However, the turbidity of all samples collected for this study was below 1.0 Nephelometric turbidity units (NTU).

Table 3.2. Water Quality Data (average ± SD) for Reclaimed Water Used for Ultrafiltration Method Development Project

Collection Site/ Experiment Use	Collection Dates	*N*	Turbidity (NTU)	pH	Specific Conductance (µS @ 25° C)	TOC (mg/L)
F. Wayne Hill-10-L Expts	6/27/06–10/23/06	9	0.108 ± 0.029	7.58 ± 0.16	470 ± 45.5	9.1 ± 3.5
F. Wayne Hill-100-L Expts	11/8/06–1/24/07	10	0.079 ± 0.024	7.53 ± 0.20	442 ± 25.1	12 ± 2.3
E. L. Huie-10-L Expts	12/5/06–1/2/07	2	0.610, 0.696	7.15, 7.40	516, 416	16, 13
E. L. Huie-100-L Expts	1/16/07–1/30/07	3	0.731 ± 0.111	7.38 ± 0.14	429 ± 53.4	12 ± 1.9

3.4 ULTRAFILTER BLOCKING EXPERIMENTS

The first set of ultrafiltration method development experiments were planned to investigate whether pretreatment (or "blocking") of the ultrafilters (UFs) results in increased microbial recovery. These experiments were performed using 10-L reclaimed water samples from the F. Wayne Hill plant (collected from the ultrafiltration facility). For each experiment, three 10-L samples were processed (one sample for each type of UF investigated). Average microbial seeding levels for these blocking experiments were: 8,500 ± 3,500 pfu (MS2); 23,000 ± 35,000 pfu (phi X174); 2400 ± 820 cfu (*C. perfringens* spores); 22,000 ± 17,000 (*E. coli*); and 120,000 ± 50,000 (*C. parvum* oocysts). Tables 3.3, 3.4, and 3.5 present data on the effectiveness of ultrafilter blocking using a 5% solution of filter-sterilized calf serum. For these experiments, blocking was performed according to Hill et al. (2007). Calf serum blocking was not associated with increased recovery of microbes when using Fresenius F200NR UFs or Baxter Exeltra Plus 210 UFs (Tables 3.3 and 3.5, respectively). Calf serum blocking of the Minntech HPH 1400 ultrafilters was associated with significantly ($p < 0.001$) higher recovery efficiencies for four microorganisms: MS2 ($p = 0.008$), phi X174 ($p = 0.005$), *E. coli* ($p = 0.03$) and *C. perfringens* ($p = 0.04$) (Table 3.4). The recovery efficiencies of *C. parvum* oocysts were not significantly different ($p > 0.05$) with or without blocking.

Table 3.3. Results of 10-L Ultrafilter Blocking Experiments Using Fresenius F200NR Filters and Reclaimed Water from the F. Wayne Hill Facility in Gwinnett County, GA

		Mean Percent Recovery ± *SD*	
Microbe	***N***	**No Blocking**	**Calf Serum Blocking**
MS2[#]	3	79 ± 18	83 ± 2.0
Phi X174[#]	3	98 ± 16	100 ± 9.0
E. coli[#]	3	64 ± 22	60 ± 14
C. perfringens spores[#]	3	90 ± 25	78 ± 25
C. parvum oocysts[#]	3	110 ± 14	100 ± 4.0

[#]$P > 0.05$, no blocking vs. calf serum blocking by two-way ANOVA analysis

Table 3.4. Results of 10-L Ultrafilter Blocking Experiments Using Minntech HPH 1400 Filters and Reclaimed Water from the F. Wayne Hill Facility in Gwinnett County, GA

		Mean Percent Recovery ± *SD*	
Microbe	***N***	**No Blocking**	**Calf Serum Blocking**
MS2*	3	42 ± 12	80 ± 6.0
Phi X174*	3	25 ± 5.0	52 ± 6.0
*E. coli**	3	19 ± 9.0	38 ± 3.0
C. perfringens spores*	3	24 ± 4.0	57 ± 20
C. parvum oocysts[#]	3	91 ± 23	94 ± 23

[#]$P > 0.05$, no blocking vs. calf serum blocking by two-way ANOVA analysis
*$P < 0.05$, no blocking vs. calf serum blocking by two-way ANOVA analysis

Table 3.5. Results of 10-L Ultrafilter Blocking Experiments Using Baxter Exeltra Plus 210 Filters and Reclaimed Water from the F. Wayne Hill Facility in Gwinnett County, GA

		Percent Recovery ± *SD*	
Microbe	***N***	**No Blocking**	**Calf Serum Blocking**
MS2[#]	3	88 ± 15	87 ± 10
Phi X174[#]	3	66 ± 12	70 ± 7.3
E. coli[#]	3	63 ± 17	75 ± 1.9
C. perfringens spores[#]	3	100 ± 23	110 ± 48
C. parvum oocysts[#]	3	120 ± 13	120 ± 18

[#]$P > 0.05$, no blocking vs. calf serum blocking by two-way ANOVA analysis

3.5 ULTRAFILTER ELUTION AND BACKFLUSHING EXPERIMENTS

Based on the results of the blocking experiments, in which little benefit from calf serum blocking was measured, a set of experiments was designed to investigate the effects of using a surfactant solution to elute the microbes off of non-blocked UFs after processing 10-L of reclaimed water. An eluent solution comprised of 0.01% Tween 80 was used for these experiments (with an eluent recirculation rate of 2000 mL/min). Tween 80 was found to be an effective surfactant for elution by Hill et al. (2007) and Polaczyk et al. (2008) and for backflushing by Hill et al. (2005). The elution procedure was performed as described by Hill et al. (2007).

For the present study, elution of UFs was investigated using two experimental procedures run in parallel: one in which retentate and eluent were collected separately, and the other in which retentate and eluent were combined and the eluted filter subsequently backwashed. The benefit of the elution procedure was quantified by separately testing the retentate samples and eluent samples. The data from this experimental protocol is presented under the heading, "Retentate Only" and "Separate Analysis of Eluent" in Tables 3.6, 3.7, and 3.8. For the same experiments, the sample handling procedure of Hill et al. (2007) was also followed, whereby the eluent sample is combined with the retentate sample to produce a final UF concentrate. The data from this experimental protocol is presented as "Retentate + Eluent" data under the heading in Tables 3.6, 3.7, and 3.8.

Although we hypothesized that the elution procedure would be sufficient to effectively desorb microbes from the hollow-fiber filter surfaces, we also wanted to evaluate whether additional filter "washing" using a backflushing procedure could result in additional desorption of microbes. Backflushing was reported by Hill et al. (2005) to be an effective technique for increasing the recovery of microbes from hollow-fiber ultrafilters. Thus, each filter that was subjected to the elution procedure was also subsequently processed using a backflushing procedure. This backflushing procedure was described by Hill et al. (2005), except that the present study used a solution of 0.5% Tween 80 and a backflushing rate of 650 mL/min. The data collected from this backflushing procedure is shown in Tables 3.6, 3.7, and 3.8 as "Backflushing of Eluted UF." Our hypothesis was that relatively little additional microbial recovery could be achieved by backflushing following elution.

The ultrafiltration-elution procedure was found to be generally associated with higher microbe recoveries versus the baseline retentate-only ultrafiltration procedure when using Fresenius filters, especially for phi X174 and *E. coli* (see Table 3.6). The effectiveness of the elution procedure was further supported by the "Separate Analysis of Eluent" data, which indicated that the elution procedure was capable of increasing microbial recovery efficiencies by an average of 17% (*C. parvum* oocysts) to 25% (*E. coli*) when using F200NR UFs. Average UF recovery efficiencies when incorporating the elution procedure (subsequently referred to as the "UF-elution procedure") were all more than 50%, which was the method performance goal of this project. The coefficients of variation (COV; SD ÷ mean) for the F200NR UF-elution recovery data were moderate (16–23%) and reasonable for an environmental microbiology procedure. With the standard deviations considered, the 95% confidence limits for the F200NR ultrafiltration-elution recovery data were more than 50%, except for *E. coli*, for which the lower bound of the 95% CL was 41%. The effectiveness of the ultrafiltration-elution procedure for F200NR UFs is further supported by the backflushing data in Table 3.6. The use of backflushing following elution did not result in substantial additional microbial recoveries, with the possible exception of *C. perfringens* spores (for which backflushing recovered an additional 10 ± 4.4% of spores). When Hill et al. (2005) used backflushing as the sole technique for desorbing microbes from hollow-fiber UFs (i.e., elution was not used), the use of Tween 80 backflushing was associated with increased recoveries of 32% for MS2 and 39% for *Bacillus globigii* spores. Thus, the elution procedure appeared to be sufficient for effective desorption of microbes adhered to the F200NR hollow fibers, and backflushing was not necessary.

Using the Minntech HPH 1400 UFs, the retentate-only, average recovery efficiencies ranged from 52% (*E. coli*) to 100% (MS2; see Table 3.7). These retentate-only recovery efficiencies were, in general, slightly lower than those achieved using the F200NR UFs, especially for phi X174 (53% for HPH 1400 vs. 73% for F200NR). The additional microbial recoveries provided by eluting the HPH 1400 UFs were 11–33%, similar to the level achieved using the F200NR UFs. The effectiveness of the UF-elution procedure for the Minntech filters was similar to the Fresenius filters. The MS2 data suggest that virus particle aggregates were present in the seed stocks used for these experiments, which would explain the estimated recovery efficiencies that were well above 100%. The COV for the HPH 1400 UF-elution recovery data were moderate for MS2, phi X174 and *C. parvum* (13%, 15%, and 14%, respectively), but were slightly higher than those observed in the F200NR experiments for *E. coli* (31%) and *C. perfringens* spores (27%). With the standard deviations considered, the 95% CLs for the HPH 1400 ultrafiltration-elution recovery data were more than 50%, except for *E. coli* and *C. perfringens* spores, for which the lower bounds of the 95% CLs were 32% and 47%, respectively (data not shown). The effectiveness of the UF-elution procedure for

F200NR UFs is further supported by the backflushing data in Table 3.7. The use of backflushing following elution did not generally result in substantial additional microbial recoveries, with the exception of *C. perfringens* spores (for which backflushing recovered an additional 15 ± 4.1% of spores).

Table 3.6. Results of 10-L Ultrafilter Elution Experiments Using Fresenius F200NR Filters and Reclaimed Water from the F. Wayne Hill Facility in Gwinnett County, GA

		Percent Recovery ± *SD*			
		Filter #1		Filter #2	
Microbe	*N*	Retentate Only	Separate Analysis of Eluent	Retentate + Eluent	Backflushing of Eluted UF
MS2[#]	6	110 ± 18	26 ± 10	120 ± 19	4.2 ± 2.2
Phi X174*	6	73 ± 13	20 ± 9.4	100 ± 20	4.5 ± 1.2
E. coli[#]	4	52 ± 18	25 ± 4.4	74 ± 17	4.4 ± 1.5
C. perfringens spores[#]	4	110 ± 37	22 ± 17	120 ± 26	10 ± 4.4
C. parvum oocysts[#]	6	92 ± 26	17 ± 16	100 ± 20	1.1 ± 0.76

[#]$P > 0.05$, retentate only vs. retentate + eluent by two-way ANOVA analysis
*$P < 0.05$, retentate only vs. retentate + eluent by two-way ANOVA analysis

Table 3.7. Results of 10-L Ultrafilter Elution Experiments Using Minntech HPH 1400 Filters and Reclaimed Water from the F. Wayne Hill Facility in Gwinnett County, GA

		Percent Recovery ± *SD*			
		Filter #1		Filter #2	
Microbe	*N*	Retentate Only	Separate Analysis of Eluent	Retentate + Eluent	Backflushing of Eluted UF
MS2*	5	100 ± 26	33 ± 14	140 ± 18	6.0 ± 2.8
Phi X174*	5	53 ± 13	23 ± 8.8	76 ± 11	6.8 ± 3.4
E. coli[#]	5	52 ± 28	33 ± 19	81 ± 25	6.6 ± 3.0
C. perfringens spores[#]	5	97 ± 46	16 ± 20	100 ± 27	15 ± 4.1
C. parvum oocysts[#]	5	95 ± 17	11 ± 8.3	97 ± 14	5.1 ± 4.8

[#]$P > 0.05$, retentate only vs. retentate + eluent by two-way ANOVA analysis
*$P < 0.05$, retentate only vs. retentate + eluent by two-way ANOVA analysis

Using the Baxter Exeltra Plus 210 UFs, the retentate-only average recovery efficiencies ranged from 50% (phi X174) to 120% (*C. perfringens* spores; see Table 3.8). These retentate-only recovery efficiencies were, in general, in the same range (50–100%) as those achieved using the F200NR and HPH 1400 UFs, but *E. coli* recoveries were higher (69% vs. 52%) for the other UFs, and MS2 recoveries were lower (80% vs. 100-110%) for the other UFs. Phi X174 recoveries were similar to those achieved using the HPH 1400 UFs (50% vs. 53%), but much lower than those achieved using the Fresenius F200NR UFs (73%). The additional microbial recoveries provided by eluting the Baxter UFs were 25–43%, levels that were higher than those achieved by eluting the F200NR and HPH 1400 UFs. The effectiveness of the UF-elution procedure was similar for the Baxter filters versus the Fresenius and Minntech filters; no statistical differences ($p = 0.24$) were found when comparing the microbial recovery effectiveness of the three filter types using the UF-elution procedure. When we further compared filter performance for each microbe separately, only for phi X174 was there a significant difference in filter performance, and the filter F200NR performed significantly better than the other two filters ($p < 0.01$). There was no significant difference between the performance of the Baxter Exeltra Plus 210 and the Minntech filter.

As noted previously for MS2 data, the *C. perfringens* data suggest that the "BioBalls" used to seed experiments with *C. perfringens* spores contained spore aggregates, despite efforts to disperse these aggregates using a procedure provided by the commercial vendor (BTF Pty, Australia). The COV for the Exeltra Plus 210 ultrafiltration-elution recovery data were low for MS2 and phi X174 (7.7 and 4.8%, respectively), moderate for *C. perfringens* spores (19%), and somewhat high for *E. coli* and *C. parvum* (26% each). With the standard deviations considered, the 95% CLs for the Exeltra Plus 210 ultrafiltration-elution recovery data were more than 50%, except for *E. coli*, for which the lower bound of the 95% CL was 47%. The effectiveness of the ultrafiltration-elution procedure for Exeltra Plus 210 UFs is further supported by the backflushing data in Table 3.8. The use of backflushing following elution did not generally result in substantial additional microbial recoveries (all less than 10%).

Table 3.8. Results of 10-L Ultrafilter Elution Experiments Using Baxter Exeltra Plus 210 Filters and Reclaimed Water from the F. Wayne Hill Facility in Gwinnett County, GA

		Percent Recovery ± *SD*			
		Filter #1		Filter #2	
Microbe	*N*	Retentate Only	Separate Analysis of Eluent	Retentate + Eluent	Backflushing of Eluted UF
MS2*	4	80 ± 14	38 ± 18	130 ± 10	6.6 ± 3.4
Phi X174[#]	4	50 ± 16	25 ± 5.6	69 ± 3.3	4.1 ± 1.7
E. coli[#]	4	69 ± 17	43 ± 15	96 ± 25	3.7 ± 2.3
C. perfringens spores[#]	4	120 ± 48	38 ± 11	140 ± 27	8.8 ± 5.6
C. parvum oocysts[#]	4	100 ± 46	31 ± 36	110 ± 29	2.1 ± 3.5

[#]$P > 0.05$, retentate only vs. retentate + eluent by two-way ANOVA analysis
*$P < 0.05$, retentate only vs. retentate + eluent by two-way ANOVA analysis

The 10-L ultrafiltration experimental results for each of the three ultrafilters investigated (i.e., F200NR, HPH 1400, and Exeltra Plus 210) showed that an ultrafiltration method incorporating elution using Tween 80, but no calf serum blocking, could be highly effective for simultaneously recovering diverse microbes from reclaimed water. This is a relatively simple technique that should be readily transferable to water utility laboratories or their contractors. The method performance goal was largely achieved for each filter using the UF-elution procedure. The only microbe for which the goal was not consistently achieved was *E. coli*; this was due primarily to relatively high COVs (23–31%) for the three filter types. However, the data from this project suggest that a 95% CL was overly ambitious for establishing a lower-bound expected method recovery of ≥ 50%. At a more appropriate 80% CL, the method performance goal was achieved for all study microbes.

3.6 COMPARING RECLAIMED WATER MATRICES FROM TWO DIFFERENT SOURCES

After the 10-L method development experiments were completed using tertiary treated wastewater from the F. Wayne Hill water reclamation facility in Gwinnett County, GA, the UF-elution method was investigated using tertiary treated wastewater from the water reclamation facility in Clayton County, GA. Secondary treated wastewater receives further treatment using a system of constructed wetlands before being discharged to a reservoir. Wastewater samples for this project were collected from the constructed wetlands discharge point (sampling location is indicated in Figure 3.4). For the experiments using the reclaimed water from Clayton County, the background concentrations of most of the study microbes were found to be sufficiently high such that microbial seeding was not necessary. The only microbe for which seeding was required was *C. parvum*. Thus, the Clayton County recovery data largely reflect the method performance effectiveness for recovery of enteric microbes naturally present in reclaimed water samples. Average background concentrations (and *C. parvum* seed levels) for these experiments are shown in Table 3.9.

Table 3.9. Microbial Levels and Recovery Efficiencies For Enteric Microbes in 10-L Samples of Reclaimed Water from Clayton County, GA, Constructed Wetlands Facility

	F+ Phages*	Somatic Phages[#]	*C. perfringens*[#]	*E. coli*[#]	*C. parvum*[#]
Background concentration/ seed level[a]	6400 ± 2100 PFU	180,000 ± 86,000 PFU	1000 ± 330 CFU	360,000 ± 210,000 CFU	180,000 ± 14,000 oocysts
F200NR	87 ± 11%	73 ± 3.3%	77 ± 28%	80 ± 16%	95 ± 5.8%
HPH 1400	130 ± 11%	92 ± 4.6%	100 ± 47%	99 ± 11%	100 ± 35%
Exeltra Plus 210	130 ± 11%	95 ± 17%	110 ± 25%	100 ± 24%	110 ± 10%

[a] Numbers shown on 100-L basis.

Note. Data shown are averages ± standard deviation

[#]$P > 0.05$ comparing recovery between three filters by one-way ANOVA analysis

*$P < 0.05$ comparing recovery between three filters by one-way ANOVA analysis

The data from the 10-L UF-elution experiments using Clayton County reclaimed water demonstrate that the UF-elution method was effective for achieving high recoveries of enteric microbes naturally present in the samples. The recovery efficiencies for each microbial parameter in Table 3.9 are similar to the measured recoveries for these filters when performing seeded experiments using 10-L wastewater samples from Gwinnett County, GA. The only microbes for which the 95% confidence limit method performance goal was not met were *C. perfringens* (measured as total *C. perfringens*, including vegetative bacteria and spores) when the F200NR and HPH 1400 filters were used (22% and 8% lower-bound 95% CL recovery efficiencies, respectively), *E. coli* (49% lower-bound 95% CL for F200NR), and *C. parvum* (31% lower-bound 95% CL when HPH 1400 filters were used). These experiments were repeated three times, so it is likely that the high standard deviations measured for these microbes would have decreased with additional replicate tests. The recovery rates differed significantly between the three filters only for F+ phage; in specific, the recovery rates for Baxter and HPH 1400 were significantly higher than F200NR ($P < 0.05$), but there was no statistically significant difference between Baxter and HPH 1400.

3.7 ULTRAFILTRATION EXPERIMENTS WITH 100-L SAMPLES OF RECLAIMED WATER

Based on the data from the 10-L experiments, 100-L samples from the F. Wayne Hill water reclamation facility in Gwinnett County were used to evaluate whether the microbial recoveries observed in the 10-L sample experiments would be maintained when larger volume samples were concentrated. The UF-elution method investigated in the 100-L experiments essentially consisted of the following procedures:

Stopping the filtration procedure at appropriate time to recover ~ 250–300 mL of the concentrated "Retentate" sample remaining in the sample container

↓

Recirculation of 500 mL of 0.01% Tween 80 solution through ultrafilter at a pumping rate of 2000 mL/min until eluent volume reduced to ~ 150 mL (point at which air bubbles begin to be drawn into the ultrafilter)

↓

Combining retentate and eluent to produce final ultrafilter concentrate sample (~400–450 mL)

↓

Assaying concentrated sample or process further, as needed

These 100-L "high seed" experiments were performed using the same seeding levels targeted in the 10-L experiments (see Table 3.10). For these 100-L Gwinnett experiments, only the F200NR and Exeltra Plus 210 ultrafilters were used in order to perform more replicate experiments for each filter type and increase the statistical power of the data comparisons between the 10-L and 100-L experiments. The F200NR experiments were performed first. In these experiments, the expected level of method performance was measured for MS2, phi X174, and *C. parvum*. Average recoveries of *C. perfringens* spores during these 100-L F200NR experiments appeared to be lower than measured during the 10-L experiments (77 ± 31% vs. 120 ± 26%), but the differences were not significant ($p = 0.10$). *E. coli* recoveries during the 100-L F200NR experiments were very low. The reason for these poor recoveries is not clear. Toxicity tests using *E. coli* and *Salmonella enterica* (serovar Typhimurium) seeded into aliquots of 100-L UF concentrate did not show any signs of bacterial die-off from exposure to the UF concentrate over a period of 3 hours. For two of the F200NR ultrafiltration experiments, *Enterococcus faecalis* was seeded into the 100-L samples as an alternative vegetative bacterial indicator organism, and good recoveries were observed (69 ± 5.1%).

Table 3.10. Microbial Seed Levels and Recovery Efficiencies for Enteric Microbes in 100-L Samples of Reclaimed Water from the F. Wayne Hill Facility in Gwinnett County, GA

	MS2	**Phi X174**	***C. perf.* spores**	***E. coli***	***E. faecalis***	***Salmonella***	***C. parvum***
Seed level	2700 ± 2200 PFU	10,000 ± 2900 PFU	2200 ± 370 CFU	13,000 ± 5700 CFU	14,000 ± 2800 CFU	15,000 ± 870 CFU	180,000 ± 16,000 oocysts
F200NR	130 ± 22%	110 ± 9.4%	77 ± 31%	0.47 ± 0.94%	69 ± 5.1%	ND	93 ± 12%
Exeltra Plus 210	110 ± 17%	110 ± 18%	110 ± 25%	2.1 ± 2.4%	89 ± 22%	21 ± 2.5%	110 ± 8.6%

Note. ND = No Data; data shown are averages ± standard deviation; for MS2, phi X174, *C. perfringens*, *E. coli* and *C. parvum*, replicate experiments were $n = 4$ (F200NR) and $n = 5$ (Exeltra); for *E. faecalis*, $n = 2$ (F200NR) and $n = 3$ (Exeltra); for *Salmonella*, $n = 3$.

After four replicate experiments using the F200NR ultrafilters yielded the same poor *E. coli* recovery, 100-L concentration experiments were performed using Baxter Exeltra Plus 210 ultrafilters. In addition to the core suite of target microorganisms, *E. faecalis* and *Salmonella enterica* (serovar Typhimurium) were seeded into the 100-L samples used for these Exeltra filter experiments. In five replicate Exeltra filter experiments, high recoveries of MS2, phi X174, *C. perfringens* spores, and *C. parvum* were observed (see Table 3.10). The recoveries for these microbes in the 100-L experiments were as high or higher than those achieved when 10-L samples were tested. In particular, phi X174 recoveries were significantly higher during the 100-L Exeltra Plus 210 recovery experiments than during the 10-L recovery experiments ($p = 0.03$). However, *E. coli* recoveries continued to be poor. *E. faecalis* and *Salmonella* were used as alternative vegetative bacterial indicator organisms for these experiments. *E. faecalis* recoveries were high (89 ± 22%), but *Salmonella* recoveries were low (21 ± 2.5%). This raises the possibility that the apparent poor performance of the UF-elution method for recovering vegetative bacteria is associated with Gram-negative bacteria but not Gram-positive bacteria. It remains unclear why this problem with *E. coli* recovery was not observed during the 10-L experiments, except for the obvious issue of increased stress of the organisms when processed during the longer 100-L ultrafiltration procedure.

Data from the 10-L experiments with the reclaimed water from the Clayton County facility showed that *E. coli* was present in this water at sufficiently high concentrations so that the recovery efficiency of the ultrafiltration procedure could be measured using naturally present *E. coli* (i.e., no laboratory seeding). Thus, to see if the apparent *E. coli* recovery problems observed when seeding the reclaimed water from the F. Wayne Hill facility in Gwinnett County would occur with reclaimed water from another type of water reclamation facility, we collected 100-L samples from the Clayton County, GA, water reclamation facility that uses constructed wetlands. For these three replicate 100-L ultrafiltration experiments (see Table 3.11), recovery efficiencies were similar to the recovery efficiencies measured using 10-L samples from the Clayton County facility (see Table 3.9). Average recovery of naturally present *E. coli* was 88 ± 13% in the 100-L Clayton County samples. The *E. coli* background levels in these samples varied widely, from 4.4×10^5 CFU/100 L to 5.6×10^6 CFU/100 L). Thus, these data demonstrated that *E. coli* could be recovered at high method efficiencies using the UF-elution procedure. These data do not, however, provide additional insights into

the cause of the apparent poor performance of the method when attempting to recover *E. coli* seeded into 100-L reclaimed water samples from the Gwinnett County facility.

Table 3.11. Microbial Levels and Recovery Efficiencies for Enteric Microbes in 100-L Samples of Reclaimed Water from Clayton County, GA, Constructed Wetlands System

	F+ Phages	Somatic Phages	*C. perfringens*	*E. coli*	*C. parvum*
Background concentration/ seed level[a]	14,000 ± 7000 PFU	$1.3 \times 10^6 \pm 1.2 \times 10^6$ PFU	14,000 ± 7000 CFU	$2.3 \times 10^6 \pm 2.9 \times 10^6$ CFU	110,000 ± 13,000 oocysts
Exeltra Plus 210	150 ± 64%	75 ± 14%	110 ± 15%	88 ± 13%	100 ± 25%

[a] Numbers shown in 100-L

Note. Data shown are averages ± standard deviation. Three replicate experiments.

3.8. LOW SEED EXPERIMENTS

After demonstrating that the UF-elution procedure was capable of achieving high recovery efficiencies for all microbe classes in 100-L samples from the Clayton County facility, experiments were designed to investigate the performance of the method for detecting low levels of the target microbes in 100-L reclaimed water samples. The experiments were designed to demonstrate detection performance using culture (for bacteria and bacteriophages), microscopy (for *C. parvum* and *Giardia intestinalis* [formerly *G. lamblia*]) and RT-PCR (for norovirus and hepatitis A virus [HAV]). For these experiments, tertiary treated wastewater from the F. Wayne Hill facility in Gwinnett County was used because this reclaimed water contained very low concentrations of background microbes. The test samples were collected following ultrafiltration at the facility, and this ultrafiltration treatment system effectively removed microbial contaminants. Fresenius F200NR UFs were used for all 100-L low-seed experiments. UF operational data and microbial seed levels for these experiments are provided in Table 3.12. In addition to the microbes shown in Table 3.12, *C. parvum* oocysts and *G. intestinalis* cysts were seeded into all low-seed experiments at a level of 200 oocysts per 100 L.

For the low-seed experiments, the ultrafilter concentrate (450 ± 17 mL) was split into three equal portions. One portion was used for membrane filtration and agar culture for the bacteria. The portion used for viral detection was concentrated further using polyethylene glycol (PEG) precipitation (12% PEG 8000, 0.9M NaCl, 1% BSA, pH 7.2). After incubating the PEG precipitation subsample for 2 hours (at 4ºC), the sample was centrifuged at 10,000 x *g* for 30 minutes. The PEG pellet was resuspended using phosphate buffered saline (PBS) containing 0.01% Tween 80 and 0.001% Antifoam Y-30 Emulsion (Sigma # A5758). Final PEG pellet concentrates were 3.7 ± 0.10 mL. The third portion of the ultrafilter concentrate was processed by centrifugation (30 minutes @ 4000 x *g* @ 4ºC), followed by immunomagnetic separation (IMS; using Aureon CG Kit) of pelleted cysts and oocysts and immunofluorescent antibody (IFA) staining (using EasyStain from BTF) for detection of Cryptosporidium and Giardia by fluorescent microscopy.

Table 3.12. UF Operational Data and Microbial Seed Levels for Low-Seed 100-L Experiments Using Reclaimed Water from the F. Wayne Hill Facility in Gwinnett County, GA

Expt. Dates	Pressure (psig)	Filtrate Flow Rate (mL/min)	MS2 Seed Level (pfu)	Phi X174 Seed Level (pfu)	HAV Seed Level (pfu)	Norovirus Seed Level (RT-PCR units)	*E. coli* Seed Level (cfu)	*E. faecalis* Seed Level (cfu)	*C. perf.* Spores Seed Level (cfu)	*Salmonella* Seed Level (cfu)
1/24/07	10	1350	235	532	100	2000	10	ND	210	539
1/31/07	10	1330	394	456	500	2000	50	320	150	372
2/7/07 to 3/1/07 (n = 4)	11 ± 1.3	1300 ± 44	386 ± 158	516 ± 348	1000	10,000	370 ± 290	430 ± 260	190 ± 28	1230 ± 394

Note. ND = No data

The data in Table 3.13 show that simultaneous detection of low levels of enteric microbes was obtained using the UF-elution method. Microbes assayed by culture or microscopy techniques were detected in all experiments. The initial seed levels of HAV and norovirus (specifically, Norwalk virus) in the experiments of January 24 and January 31 resulted in non-detects, so the seed levels were increased until positive detections were obtained. Positive detections were obtained for the last four low-seed experiments using HAV seed levels of 1000 pfu and norovirus seed levels of 10,000 RT-PCR units. To detect norovirus and HAV by RT-PCR, RNA was extracted from 140- to 500-µL volumes of PEG concentrates, and volumes of 15-25 µL of the RNA were assayed per reaction. Real-time RT-PCR for HAV was performed according to Jothikumar et al. (2005a). Real-time RT-PCR for norovirus was performed by CDC staff according to a broadly reactive assay by Jothikumar et al. (2005b) and by Emory University staff using a Norwalk virus-specific real-time RT-PCR assay by Liu et al. (Liu., et al, 2010)

Table 3.13. Detection Results for Low-Seed 100-L Experiments Using Tertiary Treated Wastewater from Gwinnett County, GA, Water Reclamation Plant

Expt. Date	MS2	Phi X174	HAV	Noro	*E. coli*	*E. faecalis*	*C. perf.* Spores	*Sal.*	*C. parvum*	*Giardia*
1/24/07	1/1[a]	1/1	0/1	0/1	1/1	ND	1/1	1/1	1/1	1/1
1/31/07	1/1	1/1	0/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1
2/7/07–3/1/07 (n = 4)	4/4	4/4	4/4	4/4	3/3	4/4	4/4	3/3	4/4	4/4

[a]"x/y" indicates microbe was detected in x out of y total samples.
Note. ND = No Data

Although the goal of the low-seed experiments was to demonstrate the performance of the UF-elution method for simultaneous detection of low levels of viruses, bacteria, and parasites in a single sample, estimated recovery efficiencies for these experiments were also calculated. For some experiments and certain analytes, the detected number of microbes was small, which limits the statistical significance of the data. Estimated recovery efficiencies for these low-seed experiments are provided for the following microbes: MS2 (59 ± 20%), phi X174 (69 ± 12%), *E. coli* (162 ± 82%), *E. faecalis* (88 ± 14%), *C. perfringens* spores (50 ± 22%), *Salmonella enterica* (serovar Typhimurium) (127 ± 18%), *C. parvum* (38 ± 22%), and *G. intestinalis* (34 ± 17%). These recovery efficiencies represent the total method efficiency, which for MS2 included UF-elution and PEG precipitation, and for the protozoa included UF-elution + centrifugation + IMS/IFA. Ultrafiltration-elution concentrates were directly assayed for bacteria culture using membrane filtration and agar culture. Because hepatitis A virus and norovirus were detected by RT-PCR, the results are reported as presence/absence. We were not able to calculate recovery efficiencies for these viruses.

CHAPTER 4

PROJECT RECOMMENDATIONS

4.1 RECOMMENDATIONS

Based on the data from the 10-L and 100-L ultrafiltration method development experiments, we recommend that reclaimed water samples of this size range can be processed to recover multiple types of microbes (viruses, bacteria, and protozoan cysts) using an ultrafiltration procedure consisting of sample recirculation through a non-blocked ultrafilter at a filtrate rate of 1.2 to 1.3 L/min, followed by filter elution using a solution of 0.01% Tween 80. When the UF retentate and eluent samples are combined for analysis, the data from this project indicate that the analysts can expect to achieve > 50% recovery of the viruses, bacteria, and protozoa present in the reclaimed water sample. For quantitative culture of bacteria, the final UF concentrate can be filtered through a standard membrane filter for agar culture. For quantitative microscopic analysis of the UF concentrate for protozoan cysts, a centrifugation procedure can be performed, followed by use of an IMS kit and IFA staining. For molecular detection of bacteria and protozoa by polymerase chain reaction or probe hybridization, the resuspended centrifugation pellet can be processed using a user-specified nucleic acid extraction procedure prior to testing. For tissue culture analysis or molecular detection of the UF concentrate for viruses, the UF concentrate can be further concentrated using a short-incubation PEG precipitation technique, then analyzed per established tissue culture or polymerase chain reaction techniques. Our recommended procedures for ultrafiltration and secondary concentration are provided in the appendix to this report.

4.2 PROBLEMS ENCOUNTERED

The primary challenge encountered in this project was the apparent poor performance of the ultrafiltration method for recovering *E. coli* **seeded** into 100-L samples of reclaimed water collected from the Gwinnett County, GA, facility. This issue required additional experiments, including side experiments to evaluate potential toxicity effects. Experiments performed to recover **naturally present** *E. coli* in 100-L samples of reclaimed water from Clayton County, GA demonstrated that the ultrafiltration method can be highly effective for recovering *E. coli* in large-volume reclaimed water samples. So, it remains unclear what the source of the apparent poor performance was for the Gwinnett samples. The problem may have been related to potential incompatibility of the laboratory *E. coli* seed stocks with processing 100-L reclaimed water samples. Two different sources of *E. coli* were used in these tests (CDC and BTF Precise Microbiology [Australia]), but both sources used the same ATCC strain of *E. coli* (ATCC 11775).

4.3 OUTREACH SUMMARY—PRESENTATIONS, PAPER, AND REPORTS

Dr. Vincent Hill presented a poster reporting results from this project at the 107th General Meeting of the American Society for Microbiology (ASM) on May 21–25, 2007 in Toronto, Canada. The ASM General Meeting draws more than 15,000 microbiologists and other scientists specializing in a diverse array of fields, including clinical microbiology, environmental microbiology, industrial health, and water and wastewater treatment engineering. Dr. Hill presented the poster for approximately two hours, during which time he

discussed this research with many scientists and public health officials interested in water and wastewater monitoring and waste management/reuse. A number of colleagues were interested in the WateReuse Research Foundation's research program, and Dr. Hill directed them to the Foundation's website for more information.

One manuscript from this study has been submitted to *Water Research*. We are waiting to hear from the editor.

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APPENDIX

Recommended ultrafiltration and secondary concentration procedures.

RECOMMENDED ULTRAFILTRATION AND

SECONDARY CONCENTRATION PROCEDURES

Materials/Vendors

a. Reagents

(1) Tween 80 (Fisher; Catalog #T164 or equivalent)

(2) Antifoam Y-30 Emulsion (Sigma; Catalog #A5758) or equivalent

(3) Polyethylene glycol (PEG) 8000 (Fisher; Catalog #BP233 or equivalent)

(4) Sodium chloride (Fisher; Catalog #S671 or equivalent)

(5) Bovine serum albumin (Sigma; Catalog #A7906 or equivalent)

b. Supplies

(1) Fresenius Optiflux F200NR Dialysis Filter (Fresenius USA; catalog #0500320E) or Minntech Hemocor HPH 1400 hemoconcentrator (Minntech, Minneapolis, MN) or Exeltra Plus 210 dialyzers (Baxter, Deerfield, IL, Cat No. 5M2132) or equivalent

(2) MasterFlex (MF) #36 silicon tubing (Cole Parmer; catalog #A-96410-36) or equivalent

(3) MasterFlex (MF) #24 silicon tubing (Cole Parmer; catalog #A-96410-24) or equivalent

(4) Masterflex tubing reducing connectors (Cole Parmer; catalog #30703-55)

(5) White hose clamps (Cole Parmer; catalog #A-06832-06 and A-06832-08 or equivalent)

(6) Flow regulator tubing clamps (Cole Parmer; catalog # A-06835-07 or equivalent)

(7) Forceps (Cole Palmer; catalog #U06443-20 or equivalent) for removing cotton filters from pipettes

(8) Scalpel, sterile, single use, #21 blade (Fisher; catalog #089275D or equivalent) to cut tubing

(9) Falcon 10ml pipets (Fisher; catalog #13-675-20) or equivalent

(10) Fresenius dialysis filter connectors (Fresenius; catalog #04-9505-1) or DIN adapters (Molded Products, Harlan, IA, Cat No. MPC-855)

c. Equipment

(1) MasterFlex Peristaltic Pump (Cole Parmer Model 7550-30 or equivalent)

(2) MasterFlex EasyLoad II Pump Head (Cole Parmer; catalog #72201-62) or equivalent

(3) Jiffy-Jack apparatus positioner (Cole Parmer; catalog #A-08057-40) or equivalent

(4) Floor model centrifuge with swinging bucket rotors capable of accepting 200–250 ml bottles or conical tubes

d. Sources

(1) Cole-Parmer Instrument Co., 625 East Bunker Court, Vernon Hills, IL 60061; www.coleparmer.com; (800) 323-4340

(2) Fresenius USA; http://www.fmcna.com; (800) 323-5188

(3) Minntech; http;//www.minntech.com; (763) 553-3307

(4) Baxter; http://www.baxter.com/products/renal/hemodialysis; (888) 736-2543

(5) Fisher Scientific; www.fishersci.com; (800) 766-7000

(6) Sigma-Aldrich; www.sigma-aldrich.com; (800) 325-3010

Disclaimer: Names of vendors or manufacturers are provided as examples of suitable product sources; inclusion does not imply endorsement by Emory University, the Centers for Disease Control and Prevention, or the Department of Health and Human Services.

Procedure

A. System Set-Up

(1) Assemble the Sample Tubing Set (sample container to ultrafilter) according to Fig. 3.2 in this report and the figure provided by Hill et al. (2007):

(a) Remove (e.g. carefully break off) the ends of a 10 ml pipette and remove cotton filter.

(b) Connect the 10-ml pipette (see Fig. 2) [to ~ 2 ft of #24 or #36 MF tubing.

(c) If using #36 tubing, run the tubing through the peristaltic pump head and connect to the 30 psig, liquid-filled pressure gauge; secure connection with a hose clamp. Then connect the pressure gauge to the influent of the ultrafilter using a short piece of #36 tubing (and secure connections with hose clamps).

(d) If using #24 tubing, connect the tubing to a short piece of #36 tubing that is placed in the peristaltic pump head using a reducing fitting. Connect the other side of the # 36 tubing to the pressure gauge, then connect the pressure gauge to the influent of the ultrafilter. Vendor-supplied connectors or "DIN Adapters" are required to attach the MF tubing to the ultrafilter. Secure connections with appropriate hose clamps.

(2) Assemble the Retentate Return Tubing Set (ultrafilter to sample container):

(a) Remove (e.g. carefully break off) the ends of a 10-ml pipette and remove cotton filter
(b) Connect the 10-ml pipette (see Fig. 2) to 2–3 ft. of #24 or 36 MF tubing.
(c) Connect #36 or #24 tubing to the effluent port of the ultrafilter using a vendor-supplied connector or DIN adapter. Secure connections with appropriate hose clamps.
(d) Attach a flow regulator tubing clamp to the MF tubing.

(3) Assemble the Filtrate Tubing Set as follows:

(a) Connect #36 MF tubing to one of the filtrate ports (the other remains capped) and run the tubing into whatever filtrate reservoir is being used.

B. Primary water sample concentration

(1) Add the sample to the sample reservoir.

(2) Set the peristaltic pump to pump at full rate (2900 mL/min) and begin pumping. Slowly adjust the flow regulator until 1.2 to 1.3 L/min is flowing out of the filtrate port at a system pressure of less than 15 psi.

(a) The concentration procedure should take no longer than 15 minutes for each 10L of sample.

(b) During the last portion of the filtration, ensure that air is not aspirated along with the sample. Adjust the sample container as needed to pool sample in a corner of the container.

(3) When the sample volume is reduced to ~250 ml, turn off the pump. (This part of the procedure will improve with analyst experience using the method.)

(4) If only one sample container is being processed, place the retentate return pipette in a sterile bottle and restart the pump to transfer as much of the UF retentate to the sterile bottle as possible.

(5) If multiple additional sample containers are being processed (e.g., two 20-L cubitainers for a 40-L total sample volume), place the retentate return pipette in the next sample container that is to be processed, then start the pump to transfer as much of the UF retentate from the previous container to the next container. Repeat process as needed, then follow previous step to transfer UF retentate to sterile bottle.

(6) Turn off the pump and open the flow regulator completely.

(7) Perform elution procedure by placing the sample tubing pipette into a bottle containing 500 mL of sterile, 0.01% Tween 80. Restart the pump at a recirculation rate of 2000 mL/min. Let eluent recirculate through the ultrafilter until ~ 75 mL remains in the eluent bottle, then remove the sample tubing pipette and hold in the air such that as much of the eluent as possible is transferred from the tubing to the eluent collection bottle. Approximately 150 mL of eluent should be collected.

(8) Combine the retentate sample and the eluent sample to produce a final UF concentrate sample of approximately 400–450 mL.

C. Sample Aliquoting and Secondary Concentration

(1) Divide sample as needed for targeted microbial analytes.

(2) For pelleting bacteria and parasites, pipette desired sample volume into 250- or 500-mL conical tube, then centrifuge for 30 minutes @ 4000 x *g* @ 4ºC. Resuspend pellet using < 50 mL of residual supernatant in the conical tubes, then transfer to a 50-mL conical tube for final spin using same centrifugation conditions. Resuspend pellet in < 5 mL of residual supernatant.

(3) To further concentrate UF sample for virus analyses, perform PEG precipitation.

(a) Add 12% PEG 8000, 0.9M NaCl, and 1% BSA to the sample aliquot being processed for virus analyses. After all reagents have dissolved, adjust pH to ~7.2 (pH 7.1 to 7.4 is acceptable).

(b) Incubate sample in 200-mL polycarbonate bottle for 2 hours at 4ºC.

(c) Centrifuge at 10,000 x *g* for 30 minutes at 4ºC.

(d) Resuspend PEG pellet phosphate buffered saline (PBS) containing 0.01% Tween 80 and 0.001% Antifoam Y-30 Emulsion. Final PEG pellet concentrate should be < 5 mL.

LITERATURE REVIEW MATRIX

of public	Authors' name	Citation	Type of water or wastewater	Volume of water	Type	Membrane type	Size (MWCO)	Filter blocking
2001	Ryan C. Kuhn, et al	Wat. Res.	deionized water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	unblocked, with bleach sanitation
			deionized water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	unblocked without bleach sanitation
			deionized water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	blocked with 10% SDS 37C 24H only
			Tap water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% FBS 20C 24h, 10% SDS 37C 24H
			Well water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% FBS 20C 24h, 10% SDS 37C 24H
			River water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% FBS 20C 24h, 10% SDS 37C 24H
1989	M. Divizia, et al	J Virol Methods	Tap water	1 L	PTGC series	Polysulfonate	10,000	flush with dechlorinated tap water
			Tap water	1 L	PTGC series	Polysulfonate	10,000	0.1% - 1% glycine
			Tap water	1 L	PTGC series	Polysulfonate	10,000	3% beef extract
			Tap water	1 L	PTGC series	Polysulfonate	10,000	flush with dechlorinated tap water
			Tap water	1 L	PTGC series	Polysulfonate	10,000	0.1% - 1% glycine
			Tap water	1 L	PTGC series	Polysulfonate	10,000	3% beef extract
			Tap water	1 L	PTGC series	Polysulfonate	10,000	flush with dechlorinated tap water
			Tap water	1 L	PTGC series	Polysulfonate	10,000	flush with dechlorinated tap water
			3% beef extract	1 L	PTGC series	Polysulfonate	10,000	3% beef extract
1995	Kevin H Oshima, et al	Can J Microbiol	Ultrapure water	500 ml	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	).3% formaldehyde, 50% glycerol solution (?
			Ultrapure water	500 ml	Hollow fiber ultrafilter	Polyacrylonitrile	13,000	).3% formaldehyde, 50% glycerol solution (?
			Ultrapure water	500 ml	Hollow fiber ultrafilter	Polyacrylonitrile	6,000	).3% formaldehyde, 50% glycerol solution (?
			Ultrapure water	500 ml	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	).3% formaldehyde, 50% glycerol solution (?
			Ultrapure water	500 ml	Hollow fiber ultrafilter	Polyacrylonitrile	13,000	).3% formaldehyde, 50% glycerol solution (?
			Ultrapure water	500 ml	Hollow fiber ultrafilter	Polyacrylonitrile	6,000	).3% formaldehyde, 50% glycerol solution (?
			Ultrapure water	500 ml	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	).3% formaldehyde, 50% glycerol solution (?
			Ultrapure water	500 ml	Hollow fiber ultrafilter	Polyacrylonitrile	13,000	).3% formaldehyde, 50% glycerol solution (?
			Ultrapure water	500 ml	Hollow fiber ultrafilter	Polyacrylonitrile	6,000	).3% formaldehyde, 50% glycerol solution (?
			Ultrapure water	500 ml	Hollow fiber ultrafilter	Polysulfonate	6,000	).3% formaldehyde, 50% glycerol solution (?
2001	L. J. Winona, et al.	Can J Microbiol	Tap water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	1% FBS 1h
			Tap water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	1% FBS 1h
			Ground water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	1% FBS 1h
			Ground water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	1% FBS 1h
			Ground water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	1% FBS 1h
			Ground water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	1% FBS 1h
			Ground water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% FBS 1h
			Ground water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% FBS 1h
			Surface water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% FBS 1h
			Surface water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% FBS 1h
			Ground water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	1% FBS 1h
			Ground water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	1% FBS 1h
			Ground water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% FBS 1h
			Ground water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% FBS 1h
			Ground water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% FBS 1h
			Ground water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	No blocking
			Ground water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	No blocking
			Surface water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	No blocking
			Surface water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	No blocking
			Surface water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	No blocking
			Surface water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% FBS 1h
			Surface water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% FBS 1h
			Surface water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% FBS 1h
			Ground water	2 L	angential flow ultrafiltratio	polyethersulfone	50,000	5% FBS 1h
			Ground water	2 L	angential flow ultrafiltratio	polyethersulfone	50,000	5% FBS 1h
			Ground water	2 L	angential flow ultrafiltratio	polyethersulfone	10,000	5% FBS 1h
			Ground water	2 L	angential flow ultrafiltratio	polyethersulfone	10,000	No blocking
			Ground water	2 L	angential flow ultrafiltratio	polyethersulfone	10,000	No blocking
			Surface water	2 L	angential flow ultrafiltratio	polyethersulfone	10,000	No blocking
			Surface water	2 L	angential flow ultrafiltratio	polyethersulfone	10,000	No blocking
			Surface water	2 L	angential flow ultrafiltratio	polyethersulfone	10,000	5% FBS 1h

of public	Authors' name	Citation	Type of water or wastewater	Volume of water	Type	Membrane type	Size (MWCO)	Filter blocking
			Surface water	2 L	angential flow ultrafiltratio	polyethersulfone	10,000	5% FBS 1h
			Surface water	2 L	angential flow ultrafiltratio	polyethersulfone	10,000	5% FBS 1h
			Surface water	2 L	angential flow ultrafiltratio	polyethersulfone	10,000	5% FBS 1h
			Surface water	2 L	angential flow ultrafiltratio	polyethersulfone	10,000	5% FBS 1h
			reagent water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	No blocking
			reagent water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	No blocking
			reagent water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	No blocking
			reagent water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	1% FBS 1h
			reagent water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	1% FBS 1h
			reagent water	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	1% FBS 1h
			reagent water	2 L	angential flow ultrafiltratio	polyethersulfone	10,000	1% FBS 1h
			reagent water	2 L	angential flow ultrafiltratio	polyethersulfone	10,000	1% FBS 1h
			reagent water	2 L	angential flow ultrafiltratio	polyethersulfone	10,000	1% FBS 1h
2005	John Olszewski, et al	Can J Microbiol	Ground water with PBS	100 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	lf serum 1h recirculation at RT, then 4C ove
			Ground water with PBS	100 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	lf serum 1h recirculation at RT, then 4C ove
			Ground water with PBS	100 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	lf serum 1h recirculation at RT, then 4C ove
			Ground water with PBS	100 L	angential flow ultrafiltratio	polyethersulfone	10,000	5% calf serum 1h
			Ground water with PBS	100 L	angential flow ultrafiltratio	polyethersulfone	10,000	5% calf serum 1h
			Ground water with PBS	100 L	angential flow ultrafiltratio	polyethersulfone	10,000	5% calf serum 1h
			Surface water with PBS	100 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	lf serum 1h recirculation at RT, then 4C ove
			Surface water with PBS	100 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	lf serum 1h recirculation at RT, then 4C ove
			Surface water with PBS	100 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	lf serum 1h recirculation at RT, then 4C ove
			Surface water with PBS	100 L	angential flow ultrafiltratio	polyethersulfone	10,000	5% calf serum 1h
			Surface water with PBS	100 L	angential flow ultrafiltratio	polyethersulfone	10,000	5% calf serum 1h
			Surface water with PBS	100 L	angential flow ultrafiltratio	polyethersulfone	10,000	5% calf serum 1h
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% fetal bovine serum RT overnight
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% fetal bovine serum RT overnight
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% fetal bovine serum RT overnight
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% fetal bovine serum RT overnight
2001	Otto D. Simmons III, et	Applied and Envi	Reagent water	10 L	esenius F80A, Hollow fibe	Polysulfone	80,000	No blocking
			Reagent water	10 L	Gelman capsule filter	polyethersulfone	1-um pore size	No blocking
			Surface water	10 L	esenius F80A, Hollow fibe	Polysulfone	80,000	No blocking
			Surface water	10 L	Gelman capsule filter	polyethersulfone	1-um pore size	No blocking
2003	Yao Yu Feng, et al	Applied and Envi	Reclaimed water	10 L	Gelman capsule filter	polyethersulfone	Not clear	No blocking
			Tap water	10 L	Gelman capsule filter	polyethersulfone	Not clear	No blocking
			Reservior water	10 L	Gelman capsule filter	polyethersulfone	Not clear	No blocking
			Tap water	10 L	Gelman capsule filter	polyethersulfone	Not clear	No blocking
2004	Donna S. Francy, et al	Applied and Envi	Surface water	5-10.5 L	esenius F80A, Hollow fibe	Polysulfone	80,000	No blocking
			reagent water	10 L	esenius F80A, Hollow fibe	Polysulfone	80,000	No blocking
2005	Vincent R Hill, et al	Applied and Envi	Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	No blocking
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	No blocking
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% FBS overnight
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% FBS overnight
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% FBS overnight
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% FBS overnight
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% FBS 1h
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% FBS 1h
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% calf serum overnight
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% calf serum overnight
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% calf serum overnight
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% calf serum overnight
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% calf serum overnight
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% calf serum overnight
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% calf serum overnight
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% calf serum overnight
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% calf serum overnight

of public	Authors' name	Citation	Type of water or wastewater	Volume of water	Type	Membrane type	Size (MWCO)	Filter blocking
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% calf serum overnight
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% calf serum overnight
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	5% calf serum overnight
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.1% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.1% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.1% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.1% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.1% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.1% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.1% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.1% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	NO
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	NO
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	NO
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	NO
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	NO
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	NO
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	NO
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)

of public	Authors' name	Citation	Type of water or wastewater	Volume of water	Type	Membrane type	Size (MWCO)	Filter blocking
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
			Tap water	10 L	esenius F80A, Hollow fibe	Polysulfone	15000 or 2000(	0.01% NAPP (sodium polyphosephate)
2002	Ryan C. Kuhn, et al	Can J Microbiol	Surface water	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	10% SDS 24 h, 5% FBS 20C 24h
			Surface water	10 L	Envirochek	Not clear	Not clear	Not clear
			Surface water	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	10% SDS 24 h, 5% FBS 20C 24h
			Surface water	10 L	Envirochek	Not clear	Not clear	Not clear
			Surface water	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	10% SDS 24 h, 5% FBS 20C 24h
1980	Donald Berman, et al	Applied and Envi	Distilled water	2 L or 4 L	PT series membrane	Not clear	100,000 NMWL	No
			Distilled water	2 L or 4 L	PT series membrane	Not clear	100,000 NMWL	1% Glycine
			Distilled water	2 L or 4 L	PT series membrane	Not clear	100,000 NMWL	Flocculated 3% beef extract
			Distilled water	2 L or 4 L	PT series membrane	Not clear	100,000 NMWL	No
			Distilled water	2 L or 4 L	PT series membrane	Not clear	100,000 NMWL	1% Glycine
			Distilled water	2 L or 4 L	PT series membrane	Not clear	10,000 NMWL	No
			Distilled water	2 L or 4 L	PT series membrane	Not clear	10,000 NMWL	1% Glycine
			Distilled water	2 L or 4 L	PT series membrane	Not clear	10,000 NMWL	1% Glycine
			Distilled water	2 L or 4 L	PT series membrane	Not clear	10,000 NMWL	Flocculated 3% beef extract
2003	Hugo A. Morales-Moral	Applied and Envi	Sterile water with PBS	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Sterile water with PBS	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Sterile water with PBS	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Sterile water with PBS	2 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Sterile water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Sterile water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Ground water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
			Surface water with PBS	10 L	Hollow fiber ultrafilter	Polyacrylonitrile	50,000	5% calf serum overnight
2003	Christobel Ferguson	Can J Microbiol	deionized water	10 L	Hollow fiber ultrafilter	Polysulfone	80,000	No
			deionized water	10 L	Hollow fiber ultrafilter	Polysulfone	80,000	No
			Surface water	10 L	Hollow fiber ultrafilter	Polysulfone	80,000	No
			Surface water	10 L	Hollow fiber ultrafilter	Polysulfone	80,000	No
1996	D. Garin	Wat. Res.	deionized water	20 L	angential flow ultrafiltratio	Polysulfonate	100,000	No
			deionized water	20 L	angential flow ultrafiltratio	Polysulfonate	100,000	No
1986	Janis Jansons, et al	Wat. Res.	Ground water	400 L	Hollow fiber ultrafilter DC3	H10p100-20	Not clear	No
1974	G. Belfort, et al	Wat. Res.	Water, no details	5 L	Hollow fiber ultrafilter	Cellulose acetate	30,000	No
			Water, no details	5 L	Hollow fiber ultrafilter	Cellulose acetate	30,000	No
1985	E. A. Bicknell	Wat. Sci. Tech.	Tap water	100 L	Hollow fiber ultrafilter	Polysulfone	10,000	No

of public	Authors' name	Citation	Type of water or wastewater	Volume of water	Type	Membrane type	Size (MWCO)	Filter blocking
1976	G. Belfort, et al	Wat. Res.	deionized water	5 L	Hollow fiber ultrafilter	Polysulfone	10,000	ashed with 0.1% enzyme active biodetergen
			deionized water	5 L	Hollow fiber ultrafilter	Polysulfone	10,000	ashed with 0.1% enzyme active biodetergen
			deionized water	50 L	Hollow fiber ultrafilter	Polysulfone	10,000	ashed with 0.1% enzyme active biodetergen
			deionized water	50 L	Hollow fiber ultrafilter	Polysulfone	10,000	ashed with 0.1% enzyme active biodetergen
			Tap water	50 L	Hollow fiber ultrafilter	Polysulfone	10,000	ashed with 0.1% enzyme active biodetergen
			Tap water	50 L	Hollow fiber ultrafilter	Polysulfone	10,000	ashed with 0.1% enzyme active biodetergen

of public	Authors' name	Elution	Backwash	Amendment of water sample	Test microbes	% recovery (SD)	Additonal information
2001	Ryan C. Kuhn, et al	No	No	No	Cryptosporidium parvum oocysts	47.8 (3.1)	
		No	No	No	Cryptosporidium parvum oocysts	29.3 (13.7)	
		No	No	No	Cryptosporidium parvum oocysts	48.1 (0.7)	
		No	No	No	Cryptosporidium parvum oocysts	64.8 (9.9)	
		No	No	No	Cryptosporidium parvum oocysts	76.6 (6.2)	
		No	No	No	Cryptosporidium parvum oocysts	81.0 (11.4)	
1989	M. Divizia, et al	No	tap water	No	Hepatitis A virus	100	
		No	0.1% - 1% glycine	No	Hepatitis A virus	100	
		No	3% beef extract	No	Hepatitis A virus	100	
		No	tap water	No	Poliovirus	10 ~ 15	
		No	tap water	No	Poliovirus	10 ~ 35	
		No	3% beef extract	No	Poliovirus	82	
		No	PBS	No	Poliovirus	15 ~ 20	
		No	3% beef extract	No	Poliovirus	40	
		No	PBS	No	Poliovirus	100	
1995	Kevin H Oshima, et al	No	No	No	phage T1	18.57 (3.41)	
		No	No	No	phage T1	13.31 (1.50)	
		No	No	No	phage T1	2.34 (1.18)	
		No	No	No	phage PP7	45.13 (8.25)	
		No	No	No	phage PP7	67.62 (3.75)	
		No	No	No	phage PP7	59.52 (8.61)	
		No	No	No	Poliovirus	nd	
		No	No	No	Poliovirus	14.17 (2.38)	
		No	No	No	Poliovirus	17.90 (4.79)	
		No	No	No	Poliovirus	27.1 (1.4)	
2001	L. J. Winona, et al.	No	No	No	phage T1	46 (24)	
		No	No	No	phage PP7	78 (18)	
		No	No	No	phage T1	12 (2)	
		No	No	No	phage PP7	37 (8)	
		10% FBS	No	No	phage T1	44 (17)	
		No	No	No	phage PP7	37 (8)	
		10% FBS	No	No	phage T1	44 (17)	
		10% FBS	No	No	phage PP7	55 (17)	
		No	No	No	phage T1	72 (9)	
		No	No	No	phage PP7	68 (12)	
		No	No	No	phage T1	6 (3)	
		No	No	No	phage PP7	32 (10)	
		10% FBS	No	No	phage T1	44 (17)	Retentate recirculation
		10% FBS	No	No	phage PP7	55 (20)	Retentate recirculation
		0.05 M glycine	No	No	phage T1	87 (3)	Retentate recirculation
		0.05 M glycine	No	No	phage PP7	88 (23)	Retentate recirculation
		0.05 M glycine	No	No	Poliovirus	90 (10)	Retentate recirculation
		0.05 M glycine	No	No	phage T1	14 (9)	Retentate recirculation
		0.05 M glycine	No	No	phage PP7	23 (11)	Retentate recirculation
		No	No	0.5% FBS added to retentate	phage T1	27 (8)	Retentate recirculation
		No	No	0.5% FBS added to retentate	phage PP7	51 (4)	Retentate recirculation
		No	No	0.5% FBS added to retentate	Poliovirus	81 (3)	Retentate recirculation
		0.05 M glycine	No	No	phage T1	61 (11)	Retentate recirculation
		0.05 M glycine	No	No	phage PP7	85 (2)	Retentate recirculation
		0.05 M glycine	No	No	Poliovirus	82 (12)	Retentate recirculation
		0.05 M glycine	No	No	phage T1	63 (27)	Immediate assay
		0.05 M glycine	No	No	phage PP7	77 (35)	Immediate assay
		0.05 M glycine	No	No	Poliovirus	55 (26)	Immediate assay
		No elution	No	0.5% FBS added to retentate	phage T1	65 (22)	Immediate assay
		No elution	No	0.5% FBS added to retentate	phage PP7	81 (16)	Immediate assay
		No elution	No	0.5% FBS added to retentate	phage T1	24 (13)	Immediate assay
		No elution	No	0.5% FBS added to retentate	phage PP7	51 (14)	Immediate assay
		0.05 M glycine	No	No	phage T1	63 (19)	Immediate assay

of public	Authors' name	Elution	Backwash	Amendment of water sample	Test microbes	% recovery (SD)	Additonal information
		0.05 M glycine	No	No	phage PP7	77 (14)	Immediate assay
		0.05 M glycine	No	No	Poliovirus	79 (13)	Immediate assay
		No elution	No	No	phage T1	51 (13)	Immediate assay
		No elution	No	No	phage PP7	81 (25)	Immediate assay
		No	No	No	phage T1	22 (25)	
		No	No	No	phage PP7	38 (30)	
		No	No	No	poliovirus	4 (5)	
		No	No	No	phage T1	47 (10)	
		No	No	No	phage PP7	94 (20)	
		No	No	No	poliovirus	98 (7)	
		No	No	No	phage T1	52 (18)	
		No	No	No	phage PP7	87 (12)	
		No	No	No	poliovirus	74 (11)	
2005	John Olszewski, et al	0.05 M glycine-NaOH	No	5 M glycine-NaOH added to retent	phage T1	71 (11)	Retentate recirculation following addition of glycine-NaOH
		0.05 M glycine-NaOH	No	5 M glycine-NaOH added to retent	phage PP7	70 (15)	Retentate recirculation following addition of glycine-NaOH
		0.05 M glycine-NaOH	No	5 M glycine-NaOH added to retent	Poliovirus 2	82 (5)	Retentate recirculation following addition of glycine-NaOH
		0.05 M glycine-NaOH	No	No	phage T1	57 (11)	Retentate recirculation following addition of glycine-NaOH
		0.05 M glycine-NaOH	No	No	phage PP7	74 (4)	Retentate recirculation following addition of glycine-NaOH
		0.05 M glycine-NaOH	No	No	Poliovirus 2	95 (15)	Retentate recirculation following addition of glycine-NaOH
		0.05 M glycine-NaOH	No	5 M glycine-NaOH added to retent	phage PP7	70 (9)	Retentate recirculation following addition of glycine-NaOH
		0.05 M glycine-NaOH	No	5 M glycine-NaOH added to retent	Poliovirus 2	86 (4)	Retentate recirculation following addition of glycine-NaOH
		0.05 M glycine-NaOH	No	5 M glycine-NaOH added to retent	phage T1	69 (18)	Retentate recirculation following addition of glycine-NaOH
		0.05 M glycine-NaOH	No	No	phage PP7	123 (25)	Retentate recirculation following addition of glycine-NaOH
		0.05 M glycine-NaOH	No	No	Poliovirus 2	104 (10)	Retentate recirculation following addition of glycine-NaOH
		0.05 M glycine-NaOH	No	No	Poliovirus 2	56 (6)	Retentate recirculation following addition of glycine-NaOH
		No	No	0 to water, 0.5 M glycine-NaOH ac	phage T1	72.9 (17)	Retentate recirculation following addition of glycine-NaOH
		No	No	0 to water, 0.5 M glycine-NaOH ac	phage PP7	61.5 (5)	Retentate recirculation following addition of glycine-NaOH
		No	No	0 to water, 0.5 M glycine-NaOH ac	phage T1	40.1% - 74.7%	Retentate recirculation following addition of glycine-NaOH
		No	No	0 to water, 0.5 M glycine-NaOH ac	phage PP7	62.5% - 76.1%	Retentate recirculation following addition of glycine-NaOH
2001	Otto D. Simmons III, et	mM PBS with 1% Lauret	No	no	Cryptosporidium parvum oocysts	Mean: 42 (24)	
		mM PBS with 1% Lauret	No	no	Cryptosporidium parvum oocysts	Mean: 46 (18)	
		mM PBS with 1% Lauret	No	no	Cryptosporidium parvum oocysts	Mean: 42 (27)	
		mM PBS with 1% Lauret	No	no	Cryptosporidium parvum oocysts	Mean: 15 (12)	
2003	Yao Yu Feng, et al	yes, not clear	No	No	Cryptosporidium parvum oocysts	12.4 (3.9)	NTU:0.17
		yes, not clear	No	No	Cryptosporidium parvum oocysts	14.4 (7.5)	NTU: 0.20
		yes, not clear	No	No	Cryptosporidium parvum oocysts	85.0 (5.2)	NTU: 5.0
		yes, not clear	No	No	Cryptosporidium parvum oocysts	14.4 (7.5)	NTU: 4.5
2004	Donna S. Francy, et al	mM PBS with 1% Lauret	No	No	Cryptosporidium parvum oocysts	Mean: 23.6	
		mM PBS with 1% Lauret	No	No	Cryptosporidium parvum oocysts	39 (28) - 79 (5)	
2005	Vincent R Hill, et al	NO	No	No	MS2	44 (30)	
		NO	No	No	Salmonella	10 (1)	
		NO	No	No	MS2	51 (19)	
		NO	No	No	Salmonella	31 (30)	
		NO	No	1% NAPP (sodium polyphosephat	MS2	108 (16)	
		NO	No	1% NAPP (sodium polyphosephat	Salmonella	49 (23)	
		NO	No	1% NAPP (sodium polyphosephat	MS2	71 (11)	
		NO	No	NO	MS2	50 (14)	
		NO	No	1% NAPP (sodium polyphosephat	MS2	84 (13)	
		NO	No	1% NAPP (sodium polyphosephat	E. coli	70 (13)	
		NO	No	1% NAPP (sodium polyphosephat	E. faecalis	71 (10)	
		NO	No	1% NAPP (sodium polyphosephat	Salmonella	62	
		NO	No	1% NAPP (sodium polyphosephat	B. globigii spores	52	
		NO	No	1% NAPP (sodium polyphosephat	Microspheres	103	
		NO	APP (sodium polyphos	1% NAPP (sodium polyphosephat	MS2	89 (15)	
		NO	APP (sodium polyphos	1% NAPP (sodium polyphosephat	E. coli	72 (13)	
		NO	APP (sodium polyphos	1% NAPP (sodium polyphosephat	E. faecalis	93 (3)	

of public	Authors' name	Elution	Backwash	Amendment of water sample	Test microbes	% recovery (SD)	Additonal information
		NO	APP (sodium polyphos	1% NAPP (sodium polyphosephat	Salmonella	79	
		NO	APP (sodium polyphos	1% NAPP (sodium polyphosephat	B. globigii spores	63	
		NO	APP (sodium polyphos	1% NAPP (sodium polyphosephat	Microspheres	110	
		NO	NO	1% NAPP (sodium polyphosephat	MS2	71 (25)	
		NO	NO	1% NAPP (sodium polyphosephat	E. coli	74 (8)	
		NO	NO	1% NAPP (sodium polyphosephat	E. faecalis	12 (4)	
		NO	NO	1% NAPP (sodium polyphosephat	Microspheres	32 (15)	
		NO	APP (sodium polyphos	1% NAPP (sodium polyphosephat	MS2	82 (25)	
		NO	APP (sodium polyphos	1% NAPP (sodium polyphosephat	E. coli	98 (8)	
		NO	APP (sodium polyphos	1% NAPP (sodium polyphosephat	E. faecalis	76 (9)	
		NO	APP (sodium polyphos	1% NAPP (sodium polyphosephat	Microspheres	93 (14)	
		NO	NO	01% NAPP (sodium polyphosepha	MS2	86 (20)	
		NO	NO	01% NAPP (sodium polyphosepha	E. coli	72 (15)	
		NO	NO	01% NAPP (sodium polyphosepha	E. faecalis	24 (5)	
		NO	NO	01% NAPP (sodium polyphosepha	Microspheres	15 (10)	
		NO	NAPP (sodium polypho	01% NAPP (sodium polyphosepha	MS2	96 (21)	
		NO	NAPP (sodium polypho	01% NAPP (sodium polyphosepha	E. coli	93 (15)	
		NO	NAPP (sodium polypho	01% NAPP (sodium polyphosepha	E. faecalis	87 (11)	
		NO	NAPP (sodium polypho	01% NAPP (sodium polyphosepha	Salmonella	65 (9)	
		NO	NAPP (sodium polypho	01% NAPP (sodium polyphosepha	B. globigii spores	46 (15)	
		NO	NAPP (sodium polypho	01% NAPP (sodium polyphosepha	Microspheres	59 (13)	
		NO	No	0.01% Tween 80	MS2	105 (23)	
		NO	No	0.01% Tween 80	E. coli	121	
		NO	No	0.01% Tween 80	E. faecalis	126	
		NO	No	0.01% Tween 80	Salmonella	80	
		NO	No	0.01% Tween 80	B. globigii spores	106	
		NO	No	0.01% Tween 80	Microspheres	116	
		NO	NAPP (sodium polypho	0.01% Tween 80	MS2	106 (23)	
		NO	NAPP (sodium polypho	0.01% Tween 80	E. coli	123	
		NO	NAPP (sodium polypho	0.01% Tween 80	E. faecalis	133	
		NO	NAPP (sodium polypho	0.01% Tween 80	Salmonella	84	
		NO	NAPP (sodium polypho	0.01% Tween 80	B. globigii spores	107	
		NO	NAPP (sodium polypho	0.01% Tween 80	Microspheres	117	
		NO	No	0.002% Tween 80	MS2	70	
		NO	No	0.002% Tween 80	E. coli	129	
		NO	No	0.002% Tween 80	E. faecalis	87	
		NO	No	0.002% Tween 80	Microspheres	100	
		NO	NAPP (sodium polypho	0.002% Tween 80	MS2	73	
		NO	NAPP (sodium polypho	0.002% Tween 80	E. coli	134	
		NO	NAPP (sodium polypho	0.002% Tween 80	E. faecalis	106	
		NO	NAPP (sodium polypho	0.002% Tween 80	Microspheres	103	
		NO	NO	NO	MS2	34 (28)	
		NO	NO	NO	Echovirus	69 (15)	
		NO	NO	NO	Salmonella	67 (2)	
		NO	NO	NO	E. faecalis	80 (24)	
		NO	NO	NO	B. globigii spores	33 (7)	
		NO	NO	NO	C. parvum	45 (36)	
		NO	NO	NO	Microspheres	81 (8)	
		NO	NAPP (sodium polypho	01% NAPP (sodium polyphosepha	MS2	59 (10)	
		NO	NAPP (sodium polypho	01% NAPP (sodium polyphosepha	Echovirus	62 (12)	
		NO	NAPP (sodium polypho	01% NAPP (sodium polyphosepha	Salmonella	63 (8)	
		NO	NAPP (sodium polypho	01% NAPP (sodium polyphosepha	E. faecalis	65 (18)	
		NO	NAPP (sodium polypho	01% NAPP (sodium polyphosepha	B. globigii spores	45 (17)	
		NO	NAPP (sodium polypho	01% NAPP (sodium polyphosepha	C. parvum	98 (17)	
		NO	NAPP (sodium polypho	01% NAPP (sodium polyphosepha	Microspheres	79 (15)	
		NO	Tween 80 + 0.01% Tw	01% NAPP (sodium polyphosepha	MS2	65 (35)	

of public	Authors' name	Elution	Backwash	Amendment of water sample	Test microbes	% recovery (SD)	Additonal information
		NO	Tween 80 + 0.01% Tw	01% NAPP (sodium polyphosepha	Echovirus	97 (58)	
		NO	Tween 80 + 0.01% Tw	01% NAPP (sodium polyphosepha	Salmonella	74 (15)	
		NO	Tween 80 + 0.01% Tw	01% NAPP (sodium polyphosepha	E. faecalis	79 (20)	
		NO	Tween 80 + 0.01% Tw	01% NAPP (sodium polyphosepha	B. globigii spores	70 (19)	
		NO	Tween 80 + 0.01% Tw	01% NAPP (sodium polyphosepha	C. parvum	97 (20)	
		NO	Tween 80 + 0.01% Tw	01% NAPP (sodium polyphosepha	Microspheres	98 (11)	
		NO	0.5-0.1% Tween 80	01% NAPP (sodium polyphosepha	MS2	91 (33)	
		NO	0.5-0.1% Tween 80	01% NAPP (sodium polyphosepha	Echovirus	49 (47)	
		NO	0.5-0.1% Tween 80	01% NAPP (sodium polyphosepha	Salmonella	70 (13)	
		NO	0.5-0.1% Tween 80	01% NAPP (sodium polyphosepha	E. faecalis	83 (13)	
		NO	0.5-0.1% Tween 80	01% NAPP (sodium polyphosepha	B. globigii spores	84 (47)	
		NO	0.5-0.1% Tween 80	01% NAPP (sodium polyphosepha	C. parvum	83 (17)	
		NO	0.5-0.1% Tween 80	01% NAPP (sodium polyphosepha	Microspheres	102 (23)	
2002	Ryan C. Kuhn, et al	NO	NO	NO	Cryptosporidium parvum oocysts	average 55.3 (25.7)	
		NO	NO	NO	Cryptosporidium parvum oocysts	71.9 (5.2)	NTU:3.9
		NO	NO	NO	Cryptosporidium parvum oocysts	74.1 (2.8)	NTU:3.9
		NO	NO	NO	Cryptosporidium parvum oocysts	0.4 (0.5)	NTU: 159
		NO	NO	NO	Cryptosporidium parvum oocysts	27.5 (3.6)	NTU: 159
1980	Donald Berman, et al	NO	NO	NO	Poliovirus 1	3.4-15.9	
		NO	NO	NO	Poliovirus 1	24.3	
		NO	NO	NO	Poliovirus 1	33.3-75.4	
		NO	NO	1% Glycine	Poliovirus 1	25.8	
		NO	NO	1% Glycine	Poliovirus 1	27.8-50.6	
		NO	NO	No	Poliovirus 1	16.9-17.8	
		NO	NO	No	Poliovirus 1	42.7	
		NO	NO	1% Glycine	Poliovirus 1	43.6-56.7	
		NO	NO	No	Poliovirus 1	49.6-76.8	
2003	Hugo A. Morales-Moral	No	No	No	E. coli, K-12	70 (13.4)	
		No	No	No	Salmonella, Serovar Enteritidis	36 (1.0)	
		No	No	0.1% Tween-80	E. coli, K-12	84 (20.8)	
		No	No	0.1% Tween-80	Salmonella, Serovar Enteritidis	72 (48)	
		No	No	No	E. coli, K-12	87 (2.3)	
		No	No	No	E. coli, XL1-B (antibiotics resistant)	80 (18)	
		No	No	0.1% Tween-80	E. coli, XL1-B (antibiotics resistant)	96 (5.6)	
		No	No	0.1% Tween-80	E. coli, XL1-B (antibiotics resistant)	89 (6.7)	NTU: 29.2
		No	No	0.1% Tween-80	E. coli, XL1-B (antibiotics resistant)	92 (5.6)	NTU: 22.8
		No	No	0.1% Tween-80	E. coli	86 (12)	
		0.05 M Glycine (final co	No	0.1% Tween-80	E. coli	95 (7.8)	
		No	No	0.1% Tween-80	T1 phage	38 (22)	
		0.05 M Glycine (final co	No	0.1% Tween-80	T1 phage	73 (17)	
		No	No	0.1% Tween-80	PPT phage	45 (55)	
		0.05 M Glycine (final co	No	0.1% Tween-80	PPT phage	62 (5)	
		0.05 M Glycine (final co	No	0.1% Tween-80 (?)	E. coli, XL1-B (antibiotics resistant)	91.6 (4.0)	NTU: 1.4-56.2
		0.05 M Glycine (final co	No	0.1% Tween-80 (?)	Cryptosporidium	31.6 (11.9)	NTU: 1.4-56.2
		0.05 M Glycine (final co	No	0.1% Tween-80 (?)	T1 phage	58.8 (16.5)	NTU: 1.4-56.2
		0.05 M Glycine (final co	No	0.1% Tween-80 (?)	PPT phage	62.8 (5.8)	NTU: 1.4-56.2
2003	Christobel Ferguson	1% Laureth-12 in 100 m	No	No	Cryptosporidium oocyst	62.6 (10.9)	
		1% Laureth-12 in 100 m	No	No	Giardia cyst	36.1 (20)	
		1% Laureth-12 in 100 m	No	No	Cryptosporidium oocyst	28.3 (24.3)	
		1% Laureth-12 in 100 m	No	No	Giardia cyst	25.6 (20.3)	
1996	D. Garin	3% beef extraxt solution	No	No	Poliovirus	99	
		3% beef extraxt solution	No	No	Echovirus	84	
1986	Janis Jansons, et al	No	10% beef extract in PE	10% beef extract in PBS	Poliovirus	21	
1974	G. Belfort, et al	No	No	No	Poliovirus-1	24.5 - 64.6	from 12 experiments
		No	Yes, no reagent, only	No	Poliovirus-1	62.5 - 110.1	from 8 experiments
1985	E. A. Bicknell	No	0.05 M Glycine, 2-3 tin	concentrates adjusted to 2% FCS	Poliovirus-2	76 (11) - 148 (28)	different seeding levels

of public	Authors' name	Elution	Backwash	Amendment of water sample	Test microbes	% recovery (SD)	Additonal information
1976	G. Belfort, et al	No	No	No	Poliovirus-1	6.42 - 54.2	4 experiments
		No	Yes, no reagent, only	No	Poliovirus-1	36.2 - 78.4	4 experiments
		No	No	No	Poliovirus-1	19 - 103.9	12 experiments
		No	Yes, no reagent, only	No	Poliovirus-1	32 - 153.7	12 experiments
		No	No	No	Poliovirus-1	9.1 - 26.46	4 experiments
		No	Yes, no reagent, only	No	Poliovirus-1	40.57 - 71.6	4 experiments

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WATEREUSE RESEARCH

1199 North Fairfax Street, Suite 410
Alexandria, VA 22314 USA
(703) 548-0880
Fax (703) 548-5085
E-mail: Foundation@WateReuse.org
www.WateReuse.org/Foundation