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Environmental Technology Verification Report

Removal of Microbial Contaminants in Drinking Water

Koch Membrane Systems, Inc. HF-82-35-PMPW™ Ultrafiltration Membrane

Prepared by

NSF International

Under a Cooperative Agreement with
U.S. Environmental Protection Agency

ETV ETV ETV

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THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

NSF International

ETV Joint Verification Statement

TECHNOLOGY TYPE:	**POINT-OF-ENTRY DRINKING WATER TREATMENT SYSTEM**
APPLICATION:	**REMOVAL OF MICROBIAL CONTAMINANTS IN DRINKING WATER**
PRODUCT NAME:	**HF-82-35-PMPW™ ULTRAFILTRATION MEMBRANE**
VENDOR:	**KOCH MEMBRANE SYSTEMS, INC.**
ADDRESS:	**850 MAIN STREET WILMINGTON, MA 01887**
PHONE:	**MAIN - 888-677-5624 CUSTOMER SERVICE – 800-343-0499**
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NSF International (NSF) manages the Drinking Water Systems (DWS) Center under the U.S. Environmental Protection Agency's (EPA) Environmental Technology Verification (ETV) Program. The DWS Center recently evaluated the performance of the Koch Membrane Systems, Inc. HF-82-35-PMPW Ultrafiltration (UF) Membrane. NSF performed all of the testing activities and also authored the verification report and this verification statement. The verification report contains a comprehensive description of the test.

EPA created the ETV Program to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and more cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized standards and testing organizations, stakeholder groups (consisting of buyers, vendor organizations, and permitters), and with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

ABSTRACT

Two Koch Membrane Systems HF-82-35-PMPW™ UF membrane cartridges were tested for removal of viruses, bacteria, and protozoan cysts at NSF's Drinking Water Treatment Systems Laboratory. The testing was conducted as part of a series of ETV verifications of the US Navy Office of Naval Research's Expeditionary Unit Water Purifier (EUWP), manufactured by Village Marine Tec. The tests reported herein served to demonstrate the performance of the Koch Membranes Targa®-10-48-35-PMC UF membrane cartridge used in the EUWP. The Targa-10-48-35-PMC is a larger version of the HF-82-35-PMPW; both cartridges are made with the same UF membrane fibers. For this verification, the HF-82-35-PMPW was operated at a target flux of 40 gallons per day per square foot (gfd), which is the operating flux of the Targa-10-48-35-PMC in the EUWP. This verification did not address long-term performance, membrane cleaning, or full-scale field maintenance and operation issues. These items will be addressed in the verification reports for the full EUWP.

The UF cartridges were challenged with approximately 5 $\log_{10}$ of the bacteriophage viruses fr and MS2, 7 to 8 $\log_{10}$ of the bacteria *Brevundimonas diminuta*, and 5.7 $\log_{10}$ of live *Cryptosporidium parvum* oocysts. The bacteria and viruses used in this study served as surrogates for pathogenic bacteria and viruses that may be introduced into drinking water through accidental or intentional contamination. Each challenge was 30 minutes in length. The membranes removed a minimum of 4.8 $\log_{10}$ of the viruses, 6.0 $\log_{10}$ of *B. diminuta*, and 5.7 $\log_{10}$ of *C. parvum*.

TECHNOLOGY DESCRIPTION

The following technology description was provided by the manufacturer and has not been verified.

The Koch HF-82-35-PMPW is 5" x 43" UF membrane cartridge. The membrane fibers are made of polysulfone, with a nominal fiber inner diameter of 0.9 millimeters. The nominal membrane surface area for the cartridge, using the fiber inner diameter, is 82 square feet. The nominal molecular weight cutoff rating for the membrane is 100,000 Daltons.

This verification was conducted as part of a series of ETV verifications of the US Navy Office of Naval Research's EUWP, manufactured by Village Marine Tec. The tests served to demonstrate the performance of the Koch Membrane Systems Targa-10-48-35-PMC UF membrane cartridge used in the EUWP. The Targa-10-48-35-PMC is a larger version of the HF-82-35-PMPW; both cartridges are made with the same UF fibers.

VERIFICATION TESTING DESCRIPTION

Test Site

The testing site was the Drinking Water Treatment Systems Laboratory at NSF in Ann Arbor, Michigan. A description of the test apparatus can be found in the test/quality assurance (QA) plan and verification report. The testing was conducted in February and March of 2006.

Methods and Procedures

The testing methods and procedures are detailed in the *Test/QA Plan for the Microbial Seeding Challenge Study of the Koch Membrane Systems HF-82-35-PMPW UF Membrane*. Two membrane cartridges were challenged with the bacteriophage viruses fr and MS2, the bacteria *B. diminuta*, and live *C. parvum* oocysts. The challenge bacteria and viruses were chosen because they are smaller than most other viruses and bacteria, and so provide a conservative estimate of performance. NSF also used a genetically engineered strain of *B. diminuta*. The NSF Microbiology Laboratory inserted into a culture of *B. diminuta* strain 19146 a gene conferring resistance to the antibiotic kanamycin (KanR *B. diminuta*). This allowed the Microbiology Laboratory to use a growth media amended with 50 micrograms per liter

(μg/L) of kanamycin to prohibit heterotrophic plate count (HPC) bacteria in the treated water samples from growing along with the kanamycin resistant *B. diminuta*.

The target challenge concentrations for each organism were as follows:

- MS2 and fr: $\geq 1x10^4$ plaque forming units per milliliter (PFU/mL);
- *B. diminuta*: $\geq 1x10^6$ colony forming units per 100 milliliters (CFU/100 mL); and
- *C. parvum*: $\geq 1x10^5$ oocysts per liter.

Prior to each challenge, an air bubble leak-check test procedure provided by Koch Membranes was conducted. Approximately five pounds per square inch, gauge (psig) of air was applied to the filtrate side of the membrane cartridge for five minutes, with the inlet port closed, but the reject port open. The degree to which air bubbles rose from the membrane fibers indicated whether any fibers were compromised. A steady stream of air bubbles would be indicative of a leak in a fiber.

The Targa-10-48-35-PMC membrane in the EUWP is operated at a flux of 40 gfd, with a reject flow rate of 10% of the feed flow. To approximate these operation conditions, the target feed flow rate for the HF-82-35-PMPW was 2.5 gallons per minute (gpm), and the target filtrate flow rate was 2.3 gpm.

The membranes were challenged with each organism for 30 minutes. Separate challenges were conducted for each organism. Feed and filtrate samples were collected for challenge organism enumeration at start-up, after 15 minutes of operation, and after 30 minutes of operation. All samples were analyzed in triplicate.

VERIFICATION OF PERFORMANCE

The results of the microbial challenges are presented below in Table VS-1. The measured triplicate feed and filtrate counts were averaged by calculating geometric means. The mean organism counts for each sample point were then averaged to give an overall mean count for the challenge. These counts were log_{10} transformed, and log reductions were calculated for each challenge.

Table VS-1. Mean Challenge Organism Reduction Data

	Feed		Cartridge 1 Filtrate			Cartridge 2 Filtrate		
Challenge	Geometric Mean (PFU/mL)	Log_{10}	Geometric Mean (PFU/mL)	Log_{10}	Log_{10} Reduction	Geometric Mean (PFU/mL)	Log_{10}	Log_{10} Reduction
fr	$6.7x10^4$	4.8	1	0.0	4.8	1	0.0	4.8
MS2	$6.7x10^4$	4.9	1	0.0	4.9	1	0.0	4.9
Challenge	Geometric Mean (CFU/100mL)	Log_{10}	Geometric Mean (CFU/100mL)	Log_{10}	Log_{10} Reduction	Geometric Mean (CFU/100mL)	Log_{10}	Log_{10} Reduction
B. diminuta	$8.2x10^7$	7.9	1	0.0	7.9	1	0.0	7.9
KanR *B. diminuta*	$5.4x10^7$	7.7	1	0.0	7.7	52	1.7	6.0
KanR *B. diminuta* retest for Unit 2	$1.2x10^7$	7.1	—	—	—	2	0.3	6.8
Challenge	Geometric Mean (oocysts/L)	Log_{10}	Geometric Mean (oocysts/L)	Log_{10}	Log_{10} Reduction	Geometric Mean (oocysts/L)	Log_{10}	Log_{10} Reduction
C. parvum	$5.3x10^5$	5.7	<1	0.0	5.7	<1	0.0	5.7

Note that the KanR *B. diminuta* challenge was conducted twice on Cartridge 2. This was due to the relatively high effluent counts measured during the first KanR *B. diminuta* challenge compared to the cartridge's performance in the other challenges. To check for any membrane integrity issue that could have caused the high effluent counts, the membranes were subjected to an air pressure decay test as

described in *ASTM D6908-03, Standard Practice for Integrity Testing of Water Filtration Membrane Systems*. The data is presented below in Table VS-2. The pressure decay rate for Cartridge 1 was measured to be 0.11 pounds per square inch, gauge, per minute (psig/min). The measured pressure decay rates for Cartridge 2 were 0.14 and 0.29 psig/min. Koch Membrane Systems provided an estimated severed fiber pressure decay rate of 2.1 psig/min for the HF-82-35-PMPW membrane, so the measured decay rates for Cartridge 2 are not indicative of a breach in membrane integrity. Also, the air bubble leak-check tests did not indicate that any membrane fibers were compromised during testing.

QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

NSF provided technical and quality assurance oversight of the verification testing as described in the verification report, including a review of 100% of the data. NSF QA personnel also conducted a technical systems audit during testing to ensure the testing was in compliance with the test plan. A complete description of the QA/QC procedures is provided in the verification report.

Original signed by Sally Gutierrez 09/22/06
Sally Gutierrez Date
Director
National Risk Management Research Laboratory
Office of Research and Development
United States Environmental Protection Agency

Original signed by Robert Ferguson 09/07/06
Robert Ferguson Date
Vice President
Water Systems
NSF International

NOTICE: Verifications are based on an evaluation of technology performance under specific, predetermined criteria and the appropriate quality assurance procedures. EPA and NSF make no expressed or implied warranties as to the performance of the technology and do not certify that a technology will always operate as verified. The end-user is solely responsible for complying with any and all applicable federal, state, and local requirements. Mention of corporate names, trade names, or commercial products does not constitute endorsement or recommendation for use of specific products. This report is not an NSF Certification of the specific product mentioned herein.

Availability of Supporting Documents
Copies of the test protocol, the verification statement, and the verification report (NSF report # NSF 06/24/EPADWCTR) are available from the following sources:

1. ETV Drinking Water Systems Center Manager (order hard copy)
   NSF International
   P.O. Box 130140
   Ann Arbor, Michigan 48113-0140

2. Electronic PDF copy
   NSF web site: http://www.nsf.org/info/etv
   EPA web site: http://www.epa.gov/etv

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Koch Membrane Systems, Inc.
HF-82-35-PMPW™ Ultrafiltration Membrane

Prepared by:

NSF International
Ann Arbor, Michigan 48105

Under a cooperative agreement with the U.S. Environmental Protection Agency

Jeffrey Q. Adams, Project Officer
National Risk Management Research Laboratory
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

Notice

The U.S. Environmental Protection Agency (USEPA), through its Office of Research and Development (ORD), has financially supported and collaborated with NSF International (NSF) under Cooperative Agreement No. R-82833301. This verification effort was supported by the Drinking Water Systems (DWS) Center, operating under the Environmental Technology Verification (ETV) Program. This document has been peer-reviewed, reviewed by NSF and USEPA, and recommended for public release.

Foreword

The U.S. Environmental Protection Agency (USEPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, USEPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory (NRMRL) is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threaten human health and the environment. The focus of the Laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research provides solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by USEPA's Office of Research and Development (ORD) to assist the user community and to link researchers with their clients.

Sally Gutierrez, Director
National Risk Management Research Laboratory

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Abbreviations and Acronyms

ANSI	American National Standards Institute
ASTM	ASTM International
ATCC	American Type Culture Collection
°C	degrees Celsius
CFU	colony forming unit
cm	centimeter
DWS	Drinking Water Systems
ETV	Environmental Technology Verification
EUWP	Expeditionary Unit Water Purifier
°F	degrees Fahrenheit
ft^2	square feet
gfd	gallons per square foot per day
gpd	gallons per day
gpm	gallons per minute
HCl	hydrochloric acid
HPC	heterotrophic plate count
in	inch(es)
KanR	Kanamycin resistant
L	liter
m	meter
mg	milligram
mL	milliliter
mm	millimeter
MWCO	molecular weight cutoff
NaOH	sodium hydroxide
ND	non-detect
nm	nanometer
NRMRL	National Risk Management Research Laboratory
NSF	NSF International (formerly known as National Sanitation Foundation)
NTU	Nephelometric Turbidity Unit
ONR	Office of Naval Research
ORD	Office of Research and Development
PBDW	phosphate-buffered dilution water
PFU	plaque forming unit
POE	point-of-entry
POU	point-of-use
psig	pounds per square inch, gauge
QA	quality assurance
QC	quality control
RO	reverse osmosis
RPD	relative percent difference
SLB	saline lactose broth
SOP	standard operating procedure

Abbreviations and Acronyms (continued)

TARDEC	Tank Automotive Research, Development, and Engineering Center
TDS	total dissolved solids
TOC	total organic carbon
TSA	tryptic soy agar
TSB	tryptic soy broth
UF	ultrafiltration
µg	microgram
µL	microliter
µm	micrometer
µS	microSieman
USEPA	U. S. Environmental Protection Agency

Acknowledgments

NSF International was responsible for all elements in the testing sequence, including collection of samples, calibration and verification of instruments, data collection and analysis, data management, data interpretation, and the preparation of this report.

The manufacturer of the equipment was:

Koch Membrane Systems, Inc.
850 Main Street
Wilmington, MA 01887
888-677-5624
info@kochmembrane.com

NSF wishes to thank the members of the expert technical panel for their assistance with development of the test plan.

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Chapter 1
Introduction

1.1 ETV Program Purpose and Operation

The U.S. Environmental Protection Agency (USEPA) has created the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and more cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized standards and testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; and with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders; conducting field or laboratory testing, collecting and analyzing data; and by preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The USEPA has partnered with NSF International (NSF) under the ETV Drinking Water Systems (DWS) Center to verify performance of drinking water treatment systems that benefit the public and small communities. It is important to note that verification of the equipment does not mean the equipment is "certified" by NSF or "accepted" by USEPA. Rather, it recognizes that the performance of the equipment has been determined and verified by these organizations under conditions specified in ETV protocols and test plans.

1.2 Purpose of Verification

Testing of the Koch Membrane Systems, Inc. HF-82-35-PMPW™ Ultrafiltration (UF) Membrane was conducted as part of the ETV verification of the US Navy Office of Naval Research's (ONR) Expeditionary Unit Water Purifier (EUWP), manufactured by Village Marine Tec. The test results serve to demonstrate the performance of the Koch TARGA®-10-48-35-PMC UF Membrane used in the EUWP. The TARGA-10-48-35-PMC membrane is a larger version of the HF-82-35-PMPW membrane. According to the manufacturer, both membranes use the same UF fibers. For this verification, the HF-82-35-PMPW membrane was operated at a flux similar to that of the TARGA-10-48-35-PMC so that the results can apply to membrane operation in the EUWP system.

This verification demonstrated the performance of the UF membrane against protozoan, bacterial and viral contaminants. Please note that this verification does not address long-term performance, or performance over the life of the membrane. This verification test did not

evaluate cleaning of the membranes, nor any other maintenance and operation. These items are covered under verification testing of the full-scale EUWP.

1.3 Development of Test/Quality Assurance (QA) Plan

The USEPA "Water Security Research and Technical Support Action Plan" (EPA, 2004) identifies the need to evaluate point-of-use (POU) and point-of-entry (POE) treatment system capabilities for removing likely contaminants from drinking water. As part of the ETV Program, NSF developed a test/QA plan for evaluating reverse osmosis (RO) drinking water treatment systems for removal of chemical and microbial contaminants. To assist in this endeavor, NSF assembled expert technical panels, which gave suggestions on a protocol design prior to development of the test/QA plan. Panel members included experts from USEPA, United States Army, and United States Centers for Disease Control and Prevention, Division of Parasitic Diseases, as well as a water utility microbiologist, a university professor, and an independent consultant in the POU drinking water treatment systems industry.

The product-specific test/QA plan for evaluating the HF-82-35-PMPW UF membrane was entitled *Test/QA Plan for the Microbial Seeding Challenge Study of the Koch Membrane Systems HF-82-35-PMPW™ UF Membrane.* The test/QA plan called for challenge tests using surrogate bacteria and viruses in place of testing with actual microorganisms of concern. However, live *Cryptosporidium parvum* oocysts were used as a protozoan challenge.

1.4 Testing Participants and Responsibilities

The ETV testing of the HF-82-35-PMPW UF membrane was a cooperative effort between the following participants:

NSF
USEPA
U.S. Navy ONR
U.S. Army Tank Automotive Research, Development, and Engineering Center (TARDEC)
U.S. Bureau of Reclamation
Koch Membrane Systems, Inc.

The following is a brief description of each of the ETV participants and their roles and responsibilities.

1.4.1 NSF International

NSF is a not-for-profit organization dedicated to public health and safety, and to protection of the environment. Founded in 1946 and located in Ann Arbor, Michigan, NSF has been instrumental in the development of consensus standards for the protection of public health and the environment. The USEPA partnered with NSF to verify the performance of drinking water treatment systems through the USEPA's ETV Program.

NSF performed all verification testing activities at its Ann Arbor location. NSF prepared the test/QA plan, performed all testing, managed, evaluated, interpreted, and reported on the data generated by the testing, and reported on the performance of the technology.

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1.4.2 U.S. Environmental Protection Agency

USEPA, through its Office of Research and Development (ORD), has financially supported and collaborated with NSF under Cooperative Agreement No. R-82833301. This verification effort was supported by the DWS Center operating under the ETV Program. This document has been peer-reviewed, reviewed by USEPA, and recommended for public release.

1.4.3 U.S. Navy ONR

The U.S. Navy ONR provided oversight of the EUWP development program.

Contact Information: Office of Naval Research
Logistics Thrust Program
Operations Technology Division
800 N. Quincy St.
Arlington, VA 22217
Contact: Major Alan Stocks
Phone: 703-696-2561
Email: stocksa@onr.navy.mil

1.4.4 U.S. Army TARDEC

The U.S. Army TARDEC provided oversight of EUWP design, construction, and testing.

Contact Information: U.S. Army TARDEC
c/o NFESC, ESC32
1100 23rd Avenue
Point Hueneme, CA 93043
Contact: Mr. Mark Miller
Phone: 805-982-1315
Email: mark.c.miller@navy.mil

1.4.5 U.S. Bureau of Reclamation

The U.S. Bureau of Reclamation was responsible for coordinating the testing of the UF membranes and the full EUWP system.

Contact Information:
U.S. Bureau of Reclamation
Denver Federal Center (D-8230)
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1.4.6 Koch Membrane Systems, Inc.

Koch Membrane Systems, Inc. manufactures the UF membranes tested, and also supplies the UF membranes for the EUWP. Koch Membrane Systems, Inc. was responsible for providing logistical and technical support, as needed.

Contact Information:
Koch Membrane Systems, Inc.
850 Main Street
Wilmington, MA 01887
Contact: Mr. John McArdle
Phone: 888-677-5624
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Chapter 2
Product Description

2.1 UF Membranes General Description

UF membranes remove contaminants from water through sieving based on the size of the membrane pores relative to the physical size of the contaminant. A common arrangement for the membranes is in hollow fibers, with the fibers "potted" in a resin. The flow of water through the fibers is typically "inside-out," where the water flows into the inside of the fibers at one end of the cartridge and then flows through the fiber wall leaving contaminants behind. UF membranes can be classified by pore size or the molecular weight cutoff (MWCO) point. Pore sizes generally range from 0.01 to 0.05 micrometer (μm). Typical MWCO points are 10,000 to 500,000 Daltons, with 100,000 being a common MWCO rating for drinking water treatment. With these specifications, UF membranes can remove viruses, bacteria, and protozoan cysts, as well as large molecules such as proteins, and suspended solids.

2.2 HF-82-35-PMPW™ Description

The Koch HF-82-35-PMPW is a 5-inch (in) by 43 in UF membrane cartridge. The membrane fibers are made of polysulfone. The membrane's specifications are presented in Table 2-1. The Koch TARGA-10-48-35-PMC membrane used in the EUWP uses the same UF fibers as the HF-82-35-PMPW.

The TARGA-10-48-35-PMC UF membranes are operated at a flux of 40 gallons per square foot per day (gfd) in the EUWP system. Therefore, the HF-82-35-PMPW membrane was operated at a similar flux during the challenges. To obtain this flux, the HF-82-35-PMPW was operated at a target filtrate flow rate of approximately 2.3 gallons per minute (gpm).

Table 2-1. HF-82-35-PMPW™ Specifications

Parameter	Specification
Dimensions:	
Nominal Fiber Inner Diameter	0.035 in (0.9 millimeters [mm])
Housing Outside Diameter	5 in (127 mm)
Cartridge Length	43 in (1092 mm)
Nominal Membrane Surface Area (Inner)	82 square feet (ft^2) (7.6 m^2)
Nominal MWCO	100,000 Daltons
Operating Limits:	
Max. Inlet Pressure	75 pounds per square inch, gauge (psig)
Max. Temperature	104°F (40°C)
Min. Temperature	34°F (1°C)
Max. Filtrate Flow	4.8 gpm
Max. Production Transmembrane Pressure	35 psig
Max. Backflush Transmembrane Pressure	20 psig

Chapter 3
Methods and Procedures

3.1 Introduction

The challenge tests followed the procedures described in the *Test/QA Plan for the Microbial Seeding Challenge Study of the Koch Membrane Systems HF-82-35-PMPW™ UF Membrane.* The challenge protocol was adapted from the *ETV Protocol for Equipment Verification Testing for Physical Removal of Microbiological and Particulate Contaminants.*

Two UF membrane cartridges were tested for removal of viruses, bacteria, and protozoa using the following organisms:

- Viruses – fr and MS2;
- Bacteria – *Brevundimonas diminuta*; and
- Protozoa – live *C. parvum* oocysts.

Individual challenges were conducted for each organism. See Section 3.4 for further discussion about the challenge organisms.

3.2 Membrane Cartridge Operation

As discussed in Chapter 2, the TARGA-10-48-35-PMC UF membranes are operated at a flux of 40 gfd in the EUWP system, so the HF-82-35-PMPW membrane was operated at approximately the same flux. To obtain this flux, the target filtrate flow rate was 2.3 gpm. The membranes were operated with a reject flow of approximately 10% of the feed, as in the EUWP. Therefore, to achieve a filtrate flow rate of 2.3 gpm, the feed flow rate was set at approximately 2.5 gpm.

The cartridges were mounted in a vertical orientation and were operated in parallel. The plumbing arrangement is shown in Figure 3-1. The feed port was located at the bottom of the cartridges, while the reject exited the top. Filtrate was drawn from the top, side port (Filtrate 1). The bottom, side port (Filtrate 2) was closed off during the challenges, but it was used to supply air for the fiber integrity check test described in Section 3.6. The cartridges arrived with 2-in openings on the top and bottom for the inlet and reject ports, and 1 1/4-in openings for the filtrate ports. Adapters were used to reduce the inlet port to 1/2-in for connection to the test rig. The reject port was reduced to 3/8 in, and a needle valve was plumbed into the reject line to control the reject flow. The filtrate ports were reduced to 1/2 in.

3.3 Test Apparatus

For the cleaning procedure (see Section 3.5) and all challenges but *C. parvum*, the cartridges were plumbed to a "tank rig" test station in the NSF Drinking Water Treatment Systems Laboratory. The tank rig uses a 500-gallon stainless steel tank or a 500-gallon polyethylene tank to hold the challenge water. See Figure 3-2 for a schematic diagram of the tank rig.

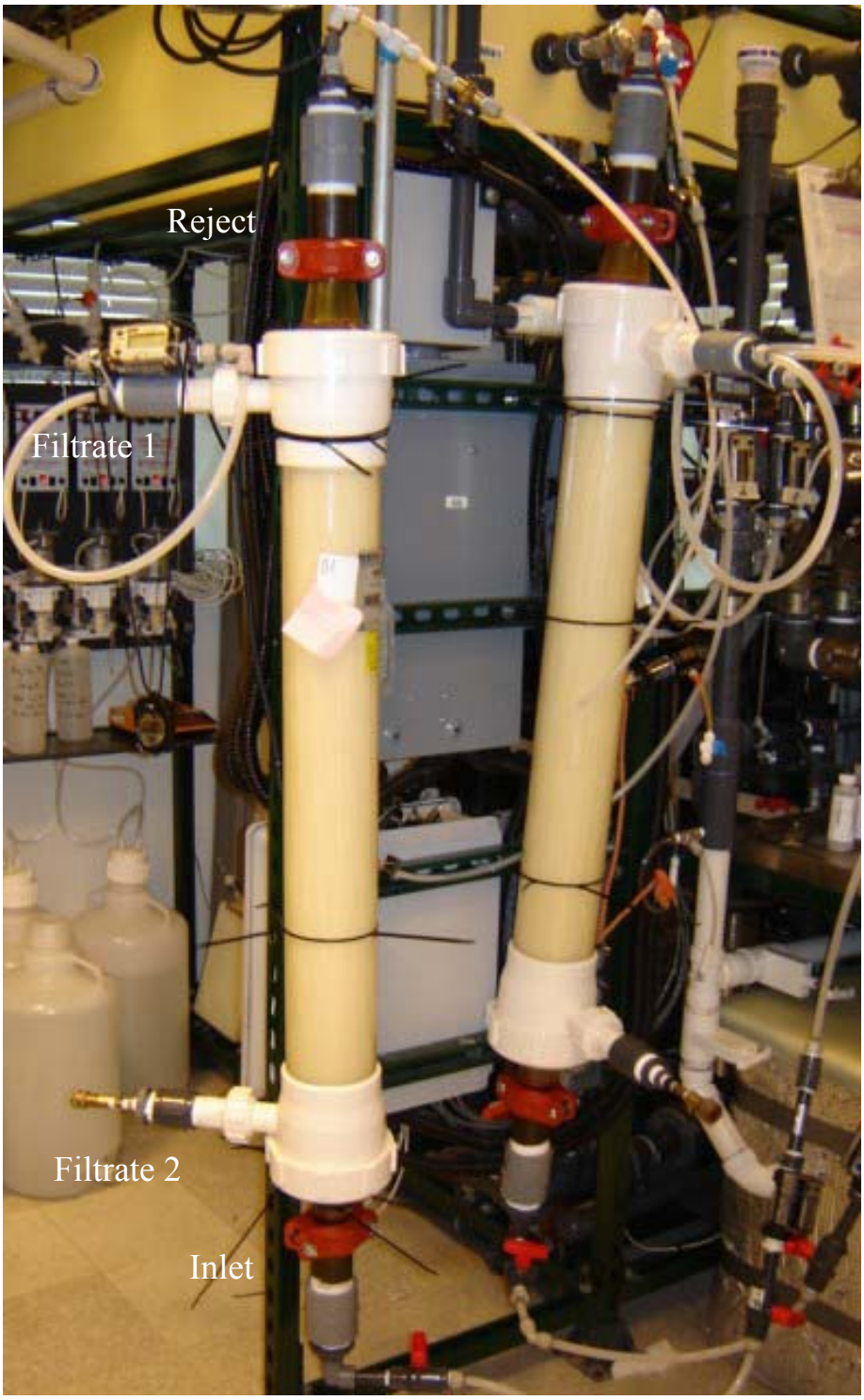

Figure 3-1. UF cartridges plumbed to injection rig test station, showing plumbing connections.

The *C. parvum* challenge was conducted using an "injection rig" in the NSF Microbiology Laboratory. This rig uses syringe pumps to inject the challenge substance directly into the feed water line upstream from the point of connection to the product being tested. The feed water, minus the challenge substance, is stored in an 80-gallon tank. Online monitors and a computer system automatically control the water chemistry. No schematic of this rig is available due to the proprietary nature of the design.

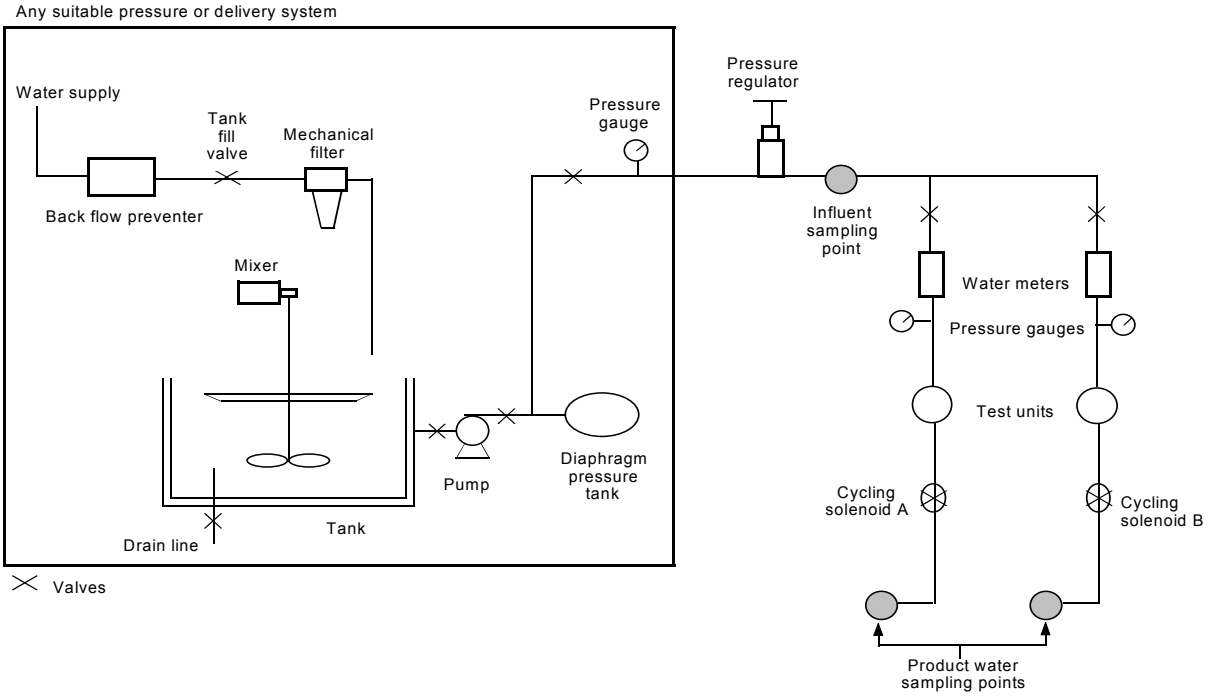

Figure 3-2. Schematic diagram of tank rig test station

3.4 Challenge Substances

3.4.1 Viruses

The virus surrogates were the coliphages fr and MS2. The American Type Culture Collection (ATCC) designation and host *E. coli* strain for each virus is given in Table 3-1.

Table 3-1. Virus and Host ATCC Designations

Virus	ATCC Designation	Host Bacteria ATCC Strain
MS2	ATCC 15597-B1	E. coli ATCC 15597
fr	ATCC 15767-B1	E. coli ATCC 19853

The viruses were chosen based on their small sizes and isoelectric points. The isoelectric point is the pH at which the virus surface is neutrally charged. MS2 is 24 nanometers (nm) in diameter with an isoelectric point at pH 3.9, and fr is 19 nm in diameter with an isoelectric point at pH 8.9. With varying isoelectric points, the viruses have different surface charges, or different strengths of negative or positive charge, depending on the pH. In solutions above the isoelectric point, the virus is negatively charged. Below the isoelectric point, the virus is positively charged.

At the challenge water pH of 7.5, MS2 should be negatively charged, while fr should be positively charged. Challenging the membranes with both viruses allowed an evaluation of whether there were any electrostatic interactions between the viruses and membrane substrate, enhancing apparent mechanical filtration.

The viruses were purchased from Biological Consulting Services of North Florida, in adequate amounts so that volumes of the suspensions received were added directly to the test water.

3.4.2 Bacteria

The bacteria surrogate was the bacteria *B. diminuta* (ATCC strain 19146). It was chosen based on its small size, as the smallest identified bacterium of concern can be as small as 0.2 μm in diameter. *B. diminuta* has a minimum diameter of 0.2 to 0.3 μm. *B. diminuta* is widely accepted as the bacteria of choice for testing filters and membranes designed to retain bacteria. It is used in the ASTM International (ASTM*) D3682-80, Standard Test Method for Retention Characteristics of 0.2-μm Membrane Filters Used in Routine Filtration Procedures for the Evaluation of Microbiological Water Quality* (2001).

The bacteria was used in its "normal" vegetative form, and also was genetically engineered by the NSF Microbiology Laboratory to be resistant to the antibiotic kanamycin. This allowed the Microbiology Laboratory to use a growth media amended with 50 micrograms per milliliter (μg/mL) of kanamycin to prohibit heterotrophic plate count (HPC) bacteria from growing along with the kanamycin resistant (KanR) *B. diminuta*. The "normal" strain and KanR strain were used in individual challenges.

B. diminuta was purchased from ATCC. The bacteria were cultivated at NSF to obtain the challenge suspensions. Section 3.8.2.2 describes the method used to create the bacteria challenges.

3.4.3 Protozoa

Live *C. parvum* oocysts were used as the protozoan challenge organism. The oocysts were purchased from Bunchgrass Farms, of Deary, Idaho. They were from a calf source with viability of greater than 50% as determined by excystation.

C. parvum oocysts have a diameter of approximately 4-6 μm. The size of *C. parvum* oocysts makes them a suitable surrogate for *Toxoplasma* oocysts (10-12 μm), and cysts of *Giardia* (7-10 μm) and *Entamoeba* (5-20 μm).

3.5 Membrane Cleaning

The UF membranes and were cleaned prior to testing in accordance with Koch Membrane Systems' "Pre-Startup Cleaning Procedure for Hollow Fiber PMPW and TARGA Cartridges." See Appendix A for the procedure.

3.6 UF Fiber Integrity Tests

Prior to each challenge, Koch Membrane Systems' air bubble leak-check test was conducted for each membrane cartridge. To perform this test, approximately five psig of air pressure was applied to the bottom filtrate port for five minutes, with the inlet port and top filtrate port closed off, but the reject port open. The reject port was clear plastic, so the degree to which bubbles rose out of the ends of the fibers could be monitored. Intermittent air bubbles due to air diffusion through the membrane were observed, but at no time did the lab technician observe a steady stream of bubbles indicative of a compromised fiber.

After all testing was complete, both membranes were subjected to an air pressure decay test as described in ASTM *D6908-03, Standard Practice for Integrity Testing of Water Filtration Membrane Systems* (2003). See Section 4.4 for the results of this test.

3.7 Microbial Challenge Test Procedure

3.7.1 Virus and Bacteria Challenge Test Water

Local tap water was treated by carbon filtration, RO, and deionization to make the base water for the tests. The base water had the following characteristics:

- Conductivity ≤ 2 microSiemens per centimeter (μS/cm) at 25°C;
- Total organic carbon (TOC) < 100 μg/liter (L);
- Total chlorine < 0.05 milligrams (mg)/L; and
- HPC bacteria < 100 colony forming units (CFU)/mL.

Of these parameters, only total chlorine was measured specifically for this verification. The other parameters are measured periodically by NSF as part of the internal quality assurance/quality control (QA/QC) program for test water quality.

The base water was adjusted to meet the following characteristics prior to addition of the challenge suspensions:

- Addition of sodium bicarbonate ($NaHCO_3$) to achieve an alkalinity (as $CaCO_3$) of 100 ± 10 mg/L prior to pH adjustment;
- pH of 7.5 ± 0.5 (the pH was adjusted if necessary with hydrochloric acid (HCl) or sodium hydroxide (NaOH)); and
- Temperature of 20 ± 2.5°C.

The test water was made fresh for each challenge. Grab samples were collected at the start of each challenge period for analysis of total chlorine, alkalinity, pH, temperature, total dissolved solids (TDS), and turbidity. pH was also measured at the end of each challenge period.

3.7.2 *C. Parvum* Challenge Test Water

Local tap water was dechlorinated with sodium thiosulfate to make the base water for the *C. parvum* challenge. Water treated by RO and deionization, as was used for the bacteria and virus challenges, was not available in the Microbiology Laboratory where the *C. parvum* challenge was conducted.

The base test water had the following characteristics prior to addition of the challenge suspensions:

- Hardness (as $CaCO_3$) ≤ 170 mg/L;
- pH of 7.5 ± 0.5 (the pH was adjusted if necessary with HCl or NaOH);
- Temperature of 20 ± 2.5 °C;
- TDS between 200 and 500 mg/L; and
- Turbidity < 1 Nephelometric Turbidity Unit (NTU).

Grab samples were collected at the start of each challenge period for analysis of total chlorine, alkalinity, pH, temperature, TDS, and turbidity. pH was also measured at the end of each challenge period.

3.7.3 Test Rig and UF Membrane Cartridge Sanitization

To keep the HPC population to a minimum, the test apparatus was sanitized prior to the first challenge test according to NSF standard operating procedure (SOP). The process is proprietary, and uses multiple chemicals as sanitizers. After the sanitization, the test apparatus was flushed until a less-than-detectable concentration of sanitizing agent was present. The UF cartridges were not sanitized prior to testing.

The UF membrane cartridges were sanitized with iodine after the *C. parvum* challenge to ensure safe handling and disposal.

3.7.4 Microbial Challenges

For the virus and bacteria challenges, suspensions of the challenge organisms were added to a tank of the test water described in 3.7.1 to create the challenge waters. The holding tank was mixed for a minimum of 30 minutes using a recirculation pump prior to beginning each test. Separate challenges were conducted for each organism. For the *C. parvum* challenge, the organism suspension was slowly drawn out of a bottle and injected into the test water described in Section 3.7.2. As described in Section 3.3, the suspension was injected directly into the feed water line upstream of the membranes. The *C. parvum* oocysts were kept in suspension throughout the challenge period by sonication. The minimum required challenge concentrations were as follows:

- MS2 and fr: ≥ $1x10^4$ plaque forming units (PFU) per milliliter;
- *B. diminuta*: ≥ $1x10^6$ CFU/100 mL; and
- *C. parvum*: ≥ $1x10^5$ oocysts/L.

All challenge concentrations exceeded the minimums. See Chapter Four for the measured concentrations.

3.7.5 Challenge Test Procedure

As discussed in Section 3.2, the cartridges were operated at a filtrate flow rate of approximately 2.3 gpm, and a reject flow of approximately 10% of the feed. To achieve this filtrate flow rate, the feed flow rate was set at approximately 2.5 gpm. This flow rate was achieved by adjusting the inlet pressure to approximately 20 psig.

Prior to addition of the challenge organism to the test water, the cartridges were flushed for at least one minute using the clean test water. During the flush, filtrate samples were collected from each cartridge for challenge chemical analysis.

After the flush, the challenge organism was added to the test water, as described in Section 3.7.4. Once the challenge water was ready, the cartridges were operated continuously for 30 minutes. Feed and filtrate samples for challenge organism analyses were collected at start-up, at 15 minutes, and at 30 minutes. Feed samples for the water chemistry parameters listed in Sections 3.7.1 and 3.7.2 were collected at the start of each challenge period. The pH was also measured at 30 minutes. The feed, filtrate, and reject flow rates were measured at start-up and 30 minutes. The inlet and filtrate flow rates were measured using Great Plains Industries 03N30 flow meters. These meters were calibrated prior to the start of testing according to NSF SOP. The reject flow rates were calculated as the feed flow rate minus the filtrate flow rate.

All challenge organism samples were analyzed in triplicate. For each sample, an appropriate volume was first collected into a sterile container, and then triplicate subsamples were drawn aseptically from this volume. The required initial sample size varied depending on the surrogate. Single samples were collected for the water chemistry parameters.

3.8 Analytical Methods

3.8.1 Water Quality Analytical Methods

The following are the analytical methods used during verification testing. All analyses followed procedures detailed in NSF SOPs.

- Alkalinity was measured according to EPA Method 310.2 with the SmartChem Discrete Analyzer. Alkalinity was expressed as mg/L $CaCO_3$.
- pH measurements were made with an Orion Model SA 720 meter. The meter was operated according to the manufacturer's instructions, which are based on Standard Method 4500-H^+.
- Water temperature was measured using an Omega model HH11 digital thermometer, or equivalent.
- TDS was measured gravimetrically using a method adapted from EPA Methods 160.3 and 160.4.
- Total chlorine was measured according to Standard Method 4500-Cl G with a Hach Model DR/2010 spectrophotometer using AccuVac vials.
- Turbidity was measured according to Standard Method 2130 using a Hach 2100N turbidimeter.

3.8.2 Microbiology Analytical Methods

3.8.2.1 Sample Processing, and Enumeration of Viruses

The viruses were enumerated using a double agar layer method published in *NSF/ANSI Standard 55 – Ultraviolet Microbiological Water Treatment Systems* for enumerating MS2. This method is similar to the double agar layer method in EPA Method 1601.

Four to eighteen hours prior to sample processing, 100 microliters (μL) of the appropriate host *E.coli* suspension was pipetted into tubes containing 10 mL of fresh tryptic soy broth (TSB), and incubated at 35°C. After incubation, 100 μL volumes of the resulting *E. coli* culture were transferred to sterile, capped test tubes.

All samples were serially diluted for enumeration, and the filtrate samples were also enumerated directly. One-mL volumes of the sample or dilution were pipetted into the *E. coli* suspension test tubes. The tubes were vortexed for a minimum of 30 seconds to "mate" the virus and host bacteria, and then 4 mL of molten, tempered TSB plus 1% agar was added to each tube. These mixtures were then poured over tryptic soy agar (TSA) plates, and allowed to solidify. The plates were incubated at 35°C for 18-24 hours. Virus plaques were counted using a Quebec Colony Counter.

3.8.2.2 *B. diminuta* Cultivation and Challenge Suspension Preparation

The bacteria was purchased from ATCC and rehydrated with nutrient broth. After 48 hours of incubation at 30°C, 5 mL of the nutrient broth culture was added to 50 mL of nutrient broth, and the resultant cultures were incubated for 48 hours at 30°C. Freezer stocks were then obtained from the nutrient broth culture, and these stocks were stored at -80°C until use.

To obtain the challenge suspensions, two 10 mL tubes of TSB were inoculated with 0.1 mL of stock culture. These tubes were incubated at 35°C for 24 hours. Then 2 mL from either tube were pipetted into eight flasks containing 1 L of Saline Lactose Broth (SLB). The eight flasks were put on a shaker and incubated in a 35°C water bath for 24 hours. The contents of all eight flasks were added to 200 gallons of base test water to create the challenge waters. The use of SLB ensures that the cells are smaller in diameter. *B. diminuta* cells grown in nutrient broth can have diameters greater than 0.5 μm. Cells grown in SLB have been measured by NSF to have diameters ranging from 0.3 to 0.5 μm.

The challenge preparation procedure was identical for both the normal *B. diminuta* and the KanR *B. diminuta*, the only difference was that for the KanR bacteria, the SLB was amended with 50 μg/L of kanamycin, and 10 μg/L of tetracycline.

3.8.2.3 Sample Processing and Enumeration of *B. diminuta*

All samples were enumerated in triplicate using a membrane filtration method based on Standard Method 9215 D. All samples were serially diluted for enumeration with sterile phosphate-buffered dilution water (PBDW), and the filtrate samples were also enumerated directly. For the

feed samples, 1 mL volumes of serial dilutions were pipetted into sterile glass vacuum filtration funnels, and 25 mL of PBDW was also poured into the funnels. For the filtrate samples, 100 mL of the straight samples and also the serial dilutions were pipetted into the funnels. The contents were then vacuum filtered through sterile 0.1 μm membrane filters. The funnels were rinsed three times with approximately 5 mL of PBDW, and the rinse water was also suctioned through the filters. The membrane filters were aseptically removed from the apparatuses and placed onto R2A agar plates. The plates were incubated at 30 °C for 48 hours. Characteristic *B. diminuta* colonies were counted with a Quebec Colony Counter.

The sample processing and enumeration procedures were identical for both the normal *B. diminuta* and the KanR *B. diminuta*, the only difference was that the R2A agar was amended with 50 μg/L of kanamycin and 10 μg/L of tetracycline for enumeration of the KanR bacteria.

3.8.2.4 Sample Processing and Enumeration of *C. parvum*

C. parvum was enumerated following EPA Method 1623 with Crypto-a-Glo™, fluorescein-labeled monoclonal antibody. The antibody is manufactured by Waterborne, Inc., and is USEPA approved for use with Method 1623. One-mL aliquots of the feed samples, or 1 L volumes of the filtrate water samples, were filtered through 0.2 μm pore size cellulose acetate filters. The numbers of oocysts retained on the filters were counted by epifluorescence microscopy with a Zeiss Axioskop 2 Plus. Please note that this method does not distinguish between viable and non-viable cysts.

Chapter 4
Results and Discussion

The virus challenges were conducted first, followed by the *B. diminuta* challenges and then the *C. parvum* challenge. After the *C. parvum* challenge, a KanR *B. diminuta* retest was conducted on Cartridge 2 (see Section 4.2 for further discussion).

The observed triplicate feed and filtrate counts were averaged by calculating geometric means. Non-detect results (<1 PFU/mL) were treated as 1 PFU/mL for the purpose of calculating the means. The geometric mean bacteria counts and $\log_{10}$ reduction data are presented in this chapter. The triplicate counts for each sample point are given in Appendix B.

As described in Section 3.6, an air bubble leak-check test protocol provided by Koch Membrane Systems, Inc. was conducted prior to each challenge to verify membrane integrity. No streams of bubbles indicating a compromised fiber were observed during any leak-check test.

4.1 Virus Reduction

Table 4-1 presents the mean virus counts in PFU/mL, the $\log_{10}$ transformations of the counts, and the log reduction calculations. The highest observed mean filtrate count was 2 PFU/mL. This count gives a minimum $\log_{10}$ reduction of 4.5 for the virus challenges. The system flush samples were all non-detect for the viruses.

The operational data and water chemistry data for the virus reduction challenges are presented below in Table 4-2. As discussed in Chapter 2, the target flux for the membranes was 40 gpd/ft^2, thus, the target filtrate flow rate was approximately 2.3 gpm. The measured filtrate flow rates ranged from 2.03 to 2.51 gpm. The flow rates varied during the challenges, depending on the inlet pressure, which in turn varied by a few psig due to operation of the feed pump.

As discussed in Section 3.4.1, fr and MS2 have different isoelectric points. Both viruses were used as challenge organisms in an attempt to determine whether electrostatic attraction played a role in virus retention by the UF membrane. The test data does not indicate that electrostatic forces played a role in virus retention.

Table 4-1. Mean Virus Counts and Log Reduction Data

Sample Point	Feed Geometric Mean (PFU/mL)	Feed Log_{10}	Cartridge 1 Filtrate Geometric Mean (PFU/mL)	Cartridge 1 Filtrate Log_{10}	Cartridge 1 Filtrate Log_{10} Reduction	Cartridge 2 Filtrate Geometric Mean (PFU/mL)	Cartridge 2 Filtrate Log_{10}	Cartridge 2 Filtrate Log_{10} Reduction
fr Challenge								
Start-up	$6.0x10^4$	4.8	<1	0.0	4.8	<1	0.0	4.8
15 Minute	$6.0x10^4$	4.8	2(1)	0.3	4.5	1(1)	0.0	4.8
30 Minute	$8.3x10^4$	4.9	1(2)	0.0	4.9	2	0.3	4.6
Overall Means	$6.7x10^4$	4.8	1	0.0	4.8	1	0.0	4.8
MS2 Challenge								
Start-up	$1.0x10^5$	5.0	<1	0.0	5.0	<1	0.0	5.0
15 Minute	$5.1x10^4$	4.7	<1	0.0	4.7	1(2)	0.0	4.7
30 Minute	$1.1x10^5$	5.0	1(2)	0.0	5.0	1(2)	0.0	5.0
Overall Means	$8.3x10^4$	4.9	1	0.0	4.9	1	0.0	4.9

(1) Triplicate count included one non-detect (<1)
(2) Triplicate counts included two non-detects (<1)

Table 4-2. Operational Data and Water Chemistry Data for Virus Challenges

Sample	fr	MS2
Cartridge 1 Feed Flow at Start-up (gpm)	2.61	2.44
Cartridge 1 Filtrate Flow at Start-up (gpm)	2.17	2.15
Cartridge 1 Reject Flow at Start-up (gpm)	0.44	0.29
Membrane Flux (gpd/ft^2)	38.1	37.8
Cartridge 2 Feed Flow at Start-up (gpm)	2.61	2.42
Cartridge 2 Filtrate Flow at Start-up (gpm)	2.33	2.35
Cartridge 2 Reject Flow at Start-up (gpm)	0.28	0.07
Membrane Flux (gpd/ft^2)	40.9	41.3
Cartridge 1 Feed Flow at 30 Minutes (gpm)	2.51	2.59
Cartridge 1 Filtrate Flow at 30 Minutes (gpm)	2.03	2.31
Cartridge 1 Reject Flow at 30 Minutes (gpm)	0.48	0.28
Membrane Flux (gpd/ft^2)	35.6	40.6
Cartridge 2 Feed Flow at 30 Minutes (gpm)	2.53	2.58
Cartridge 2 Filtrate Flow at 30 Minutes (gpm)	2.30	2.51
Cartridge 2 Reject Flow at 30 Minutes (gpm)	0.23	0.07
Membrane Flux (gpd/ft^2)	40.4	44.1
Inlet Pressure at Start-up (psig)	25	20
Inlet Pressure at 30 Minutes (psig)	20	23
Feed Water Chemistry at Start-up		
Alkalinity (mg/L $CaCO_3$)	72	77
pH	7.5	7.8
Temperature (°C)	21	22
Total Chlorine (mg/L)	Non-Detect (ND) (0.05)	ND (0.05)
TDS (mg/L)	78	83
Turbidity (NTU)	ND (0.1)	0.3
Feed Water pH at 30 Minutes	Not measured	7.9

4.2 *B. diminuta* Reduction

Table 4-3 presents the mean bacteria counts and log reduction numbers for the *B. diminuta* challenges. Cartridge 2 had filtrate counts of 1 or < 1 CFU/100mL for the "normal" *B. diminuta* challenge, but then for the KanR *B. diminuta* challenge had a mean filtrate count of 110 CFU/100mL at start-up, which then decreased to 37 and 35 CFU/100 mL at 15 and 30 minutes, respectively. The membrane integrity tests before the KanR *B. diminuta* challenge and the next challenge for *C. parvum* reduction did not indicate that the integrity of the membrane has been compromised. Also, both bacteria challenges were conducted on the same day, only a few hours apart. The "normal" *B. diminuta* challenge first, then the KanR *B. diminuta* challenge. Therefore, the KanR *B. diminuta* in the filtrate samples may have been introduced by contamination of the sample or analytical equipment instead of passing through the membranes into the filtrate. Despite the bacteria in the filtrate samples, the minimum log reduction was still 5.7. The system flush samples for these two *B. diminuta* challenges were non-detect for the bacteria.

Table 4-3. Mean *B. diminuta* Counts and Log Reduction Data

	Feed		Cartridge 1 Filtrate			Cartridge 2 Filtrate		
Sample Point	Geometric Mean (CFU/100 mL)	Log_{10}	Geometric Mean (CFU/100 mL)	Log_{10}	Log_{10} Reduction	Geometric Mean (CFU/100 mL)	Log_{10}	Log_{10} Reduction
B. diminuta								
Start-up	$8.0x10^7$	7.9	1[1]	0.0	7.9	1[1]	0.0	7.9
15 Minute	$8.6x10^7$	7.9	1[1]	0.0	7.9	<1	0.0	7.9
30 Minute	$7.9x10^7$	7.9	2	0.3	7.6	1[1]	0.0	7.9
Overall Means	$8.2x10^7$	7.9	1	0.0	7.9	1	0.0	7.9
KanR *B. diminuta*								
Start-up	$6.0x10^7$	7.8	<1	0.0	7.8	110	2.1	5.7
15 Minute	$4.5x10^7$	7.7	<1	0.0	7.7	37	1.6	6.1
30 Minute	$5.9x10^7$	7.8	1[1]	0.0	7.8	35	1.5	6.3
Overall Means	$5.4x10^7$	7.7	1	0.0	7.7	52	1.7	6.0
KanR *B. diminuta* retest for Cartridge 2								
Start-up	$1.2x10^7$	7.1	—	—	—	—[2]	—[2]	—[2]
15 Minute	$1.2x10^7$	7.1	—	—	—	1[3]	0.0	7.1
30 Minute	$1.2x10^7$	7.1	—	—	—	6	0.8	6.3
Overall Means	$1.2x10^7$	7.1	—	—	—	2	0.3	6.8

(1) Triplicate counts included two non-detects (<1)
(2) Samples purposefully not collected
(3) Triplicate count included one non-detect (<1)

Cartridge 2 was challenged again with KanR *B. diminuta* after the *C. parvum* challenge. For the second challenge, filtrate samples were not collected until 15 minutes of operation to allow the cartridge to reach a steady state of operation. KanR *B. diminuta* was found again in the filtrate samples, but at very low concentrations. It is possible that the observed CFU counts could be surviving bacteria already on the filtrate side of the membrane from the first challenge, even though the cartridge was operated for 30 minutes for the *C. parvum* challenge, and it was also sanitized with iodine to kill the *C. parvum* oocysts. The flush sample collected prior to the start

of the challenge was positive for KanR *B. diminuta*, with triplicate analysis counts of 2, 2, and 3 CFU/100 mL. The minimum calculated log reduction for the KanR. *B. diminuta* retest is 6.3 log_{10}.

Presented in Table 4-4 are the operational data and water chemistry data for the *B. diminuta* challenges. The measured filtrate flow rates for these challenges ranged from 1.97 gpm to 2.34 gpm. Most of the measured flow rates were below 2.3 gpm. Note that the alkalinity and TDS for the KanR *B. diminuta* retest are both non-detects. This could be due to a sampling error. Instead of the test water, deionized water samples may have been collected from the wrong sample port. The test logbook indicates that sodium bicarbonate was added to the tank of challenge water, and the pH of the challenge water indicates that the buffering capacity of the sodium bicarbonate was present.

Table 4-4. Operational Data and Water Chemistry Data for *B. diminuta* Challenges

Sample	*B. diminuta*	KanR *B. diminuta*	KanR *B. diminuta* Retest
Cartridge 1 Feed Flow at Start-up (gpm)	2.40	2.50	—
Cartridge 1 Filtrate Flow at Start-up (gpm)	1.97	2.03	—
Cartridge 1 Reject Flow at Start-up (gpm)	0.43	0.47	—
Membrane Flux (gpd/ft^2)	34.6	35.6	—
Cartridge 2 Feed Flow at Start-up (gpm)	2.31	2.39	2.49
Cartridge 2 Filtrate Flow at Start-up (gpm)	2.18	2.30	2.44
Cartridge 2 Reject Flow at Start-up (gpm)	0.13	0.09	0.05
Membrane Flux (gpd/ft^2)	38.3	40.4	42.8
Cartridge 1 Feed Flow at 30 Minutes (gpm)	2.49	2.45	—
Cartridge 1 Filtrate Flow at 30 Minutes (gpm)	2.02	1.98	—
Cartridge 1 Reject Flow at 30 Minutes (gpm)	0.47	0.47	—
Membrane Flux (gpd/ft^2)	35.5	34.8	—
Cartridge 2 Feed Flow at 30 Minutes (gpm)	2.40	2.32	2.38
Cartridge 2 Filtrate Flow at 30 Minutes (gpm)	2.34	2.23	2.25
Cartridge 2 Reject Flow at 30 Minutes (gpm)	0.06	0.09	0.13
Membrane Flux (gpd/ft^2)	41.1	39.2	39.5
Inlet Pressure at Start-up (psig)	19	20	20
Inlet Pressure at 30 Minutes (psig)	20	19	22
Feed Water Chemistry at Start-up			
Alkalinity (mg/L $CaCO_3$)	77	72	ND (5)
pH	7.6	7.5	7.4
Temperature (°C)	21	22	21
Total Chlorine (mg/L)	ND (0.05)	ND (0.05)	ND (0.05)
TDS (mg/L)	75	72	ND (5.0)
Turbidity (NTU)	ND (0.1)	ND (0.1)	0.1
pH at 30 Minutes	7.7	7.4	7.5

4.3 *C. parvum* Reduction

Table 4-5 presents the mean *C. parvum* counts and log reduction numbers. All filtrate samples were non-detect for *C. parvum* oocysts, giving a mean log_{10} reduction of 5.7. The system flush samples were non-detect for oocysts.

Table 4-6 presents the operational data and water chemistry data for the challenge. The measured filtrate flow rates ranged from 1.99 to 2.15 gpm, giving membrane fluxes of 34.9 to 37.8 gpd/ft^2.

Table 4-5. Mean *C. parvum* Counts and Log Reduction Data

	Feed		Cartridge 1 Filtrate			Cartridge 2 Filtrate		
Sample Point	Geometric Mean (cysts/L)	Log_{10}	Geometric Mean (cysts/L)	Log_{10}	Log_{10} Reduction	Geometric Mean (cysts/L)	Log_{10}	Log_{10} Reduction
Start-up	$4.5x10^5$	5.7	<1	0.0	5.7	<1	0.0	5.7
15 Minute	$6.3x10^5$	5.8	<1	0.0	5.8	<1	0.0	5.8
30 Minute	$5.1x10^5$	5.7	<1	0.0	5.7	<1	0.0	5.7
Overall Means	$5.3x10^5$	5.7	<1	0.0	5.7	<1	0.0	5.7

Table 4-6. Operational Data and Water Chemistry Data for *C. parvum* Challenge

Sample	Value
Cartridge 1 Feed Flow at Start-up (gpm)	2.23
Cartridge 1 Filtrate Flow at Start-up (gpm)	1.99
Cartridge 1 Reject Flow at Start-up (gpm)	0.24
Membrane Flux (gpd/ft^2)	34.9
Cartridge 2 Feed Flow at Start-up (gpm)	2.17
Cartridge 2 Filtrate Flow at Start-up (gpm)	2.11
Cartridge 2 Reject Flow at Start-up (gpm)	0.06
Membrane Flux (gpd/ft^2)	37.1
Cartridge 1 Feed Flow at 30 Minutes (gpm)	2.41
Cartridge 1 Filtrate Flow at 30 Minutes (gpm)	2.15
Cartridge 1 Reject Flow at 30 Minutes (gpm)	0.26
Membrane Flux (gpd/ft^2)	37.8
Cartridge 2 Feed Flow at 30 Minutes (gpm)	2.12
Cartridge 2 Filtrate Flow at 30 Minutes (gpm)	2.07
Cartridge 2 Reject Flow at 30 Minutes (gpm)	0.05
Membrane Flux (gpd/ft^2)	36.4
Inlet Pressure at Start-up (psig)	17
Inlet Pressure at 30 Minutes (psig)	20
Feed Water Chemistry at Start-up	
Alkalinity (mg/L $CaCO_3$)	62
pH	7.1
Temperature (°C)	20
Total Chlorine (mg/L)	ND (0.05)
TDS (mg/L)	250
Turbidity (NTU)	0.2
pH at 30 Minutes	7.1

4.4 Pressure Decay Tests

Because KanR *B. diminuta* were detected in the Cartridge 2 filtrate during the first KanR *B. diminuta* challenge, the membranes were subjected to an air pressure decay test as described in *ASTM D6908-03, Standard Practice for Integrity Testing of Water Filtration Membrane Systems*. To perform this test, the filtrate sides of the membrane cartridges were drained, and approximately 15 psig of air was applied to the membranes. The test was conducted on Cartridge 2 both with and without the water also drained from the feed side. For Cartridge 1, the test was only conducted with both sides of the membrane drained.

The test was conducted three times on Cartridge 2. The first time the filtrate side was drained, but the water remained on the feed side. The cartridge was then flushed for approximately five minutes, and the test re-run, with water still remaining on the feed side. For the third test, the feed side was drained prior to the test. For all three tests, the filtrate side air pressure was recorded at time zero, two minutes, five minutes, and seven minutes. The average pressure decay rates are presented below in Table 4-7. Koch Membrane Systems, Inc. provided an estimated severed fiber pressure decay rate of 2.1 psig/min for the HF-82-35-PMPW membrane, so the measured decay rates are not indicative of a breach in membrane integrity.

Table 4-7. Pressure Decay Test Results

Cartridge Number	Conditions	Mean Decay Rate (psig/min)
Cartridge 1	Both feed and filtrate drained	0.11
Cartridge 2	Only filtrate drained	0.29
Cartridge 2	Flushed for five minutes, filtrate side drained	0.14
Cartridge 2	Both sides drained	0.29

Chapter 5
Quality Assurance/Quality Control

5.1 Introduction

An important aspect of verification testing is the QA/QC procedures and requirements. Careful adherence to the procedures ensured that the data presented in this report was of sound quality, defensible, and representative of the equipment performance. The primary areas of evaluation were representativeness, accuracy, precision, and completeness.

Because this ETV was conducted at the NSF testing lab, all laboratory activities were conducted in accordance with the provisions of the *NSF International Laboratories Quality Assurance Manual* (NSF 2004).

5.2 Test Procedure QA/QC

NSF testing laboratory staff conducted the tests by following a USEPA-approved test/QA plan created specifically for this verification. NSF QA Department staff performed an informal audit during testing to ensure the proper procedures were followed. The audit yielded no significant findings.

As discussed in Section 4.2, the system flush sample for the KanR *B. diminuta* challenge retest was positive for the challenge organism. However, NSF does not believe that the positive system flush sample invalidates the challenge test. It does call into question whether the filtrate sample CFU counts were bacteria that went through the membrane, or whether they were already on the filtrate side from the previous challenge. Even if the *B. diminuta* cells did pass through the membrane, the challenge still demonstrated greater than 6 $\log_{10}$ reduction of *B. diminuta*.

5.3 Sample Handling

All samples analyzed by the NSF Chemistry and Microbiology Laboratories were labeled with unique ID numbers. These ID numbers appear in the NSF laboratory reports for the tests. All samples were analyzed within allowable holding times.

5.4 Chemistry Analytical Methods QA/QC

The calibrations of all analytical instruments and the analyses of all parameters complied with the QA/QC provisions of the *NSF International Laboratories Quality Assurance Manual* (NSF, 2004).

The NSF QA/QC requirements are all compliant with those given in the EPA method or Standard Method for the parameter. Also, every analytical instrument has an NSF SOP governing its use.

5.5 Microbiology Laboratory QA/QC

5.5.1 Growth Media Positive Controls

All media were checked for sterility and positive growth response when prepared and when used for microorganism enumeration. The media was discarded if growth occurred on the sterility check media, or if there was an absence of growth in the positive response check. Both *E. coli* hosts for the viruses were plated on TSA and incubated with the virus enumeration plates during sample enumeration as a second positive growth control. *B. diminuta* from the stock cultures was plated on R2A agar and incubated with the bacteria enumeration plates as a positive control.

5.5.2 Negative Controls

For each sample batch processed, an unused membrane filter and a blank with 100 mL of PBDW filtered through the membrane were also placed onto the appropriate media and incubated with the samples as negative controls. No growth was observed on any blanks.

5.6 Documentation

All laboratory activities were documented using specially prepared laboratory bench sheets and NSF laboratory reports. Data from the bench sheets and laboratory reports were entered into Excel spreadsheets. These spreadsheets were used to calculate average feeds and filtrates, and $\log_{10}$ reductions for each challenge. One hundred percent of the data entered into the spreadsheets was checked by a reviewer to confirm all data and calculations were correct.

5.7 Data Review

NSF QA/QC staff reviewed the raw data records for compliance with QA/QC requirements. NSF ETV staff checked 100% of the data in the NSF laboratory reports against the lab bench sheets.

5.8 Data Quality Indicators

The quality of data generated for this ETV is established through four indicators of data quality: representativeness, accuracy, precision, and completeness.

5.8.1 Representativeness

Representativeness refers to the degree to which the data accurately and precisely represent the expected performance of the UF membranes under normal use conditions. The membranes were operated at fluxes similar to those they face in the full EUWP system. The test water was of very low turbidity to minimize the potential for microbial adhesion to suspended particles, which could enhance log reduction. The challenge viruses and bacteria were chosen because of their small size.

Representativeness was ensured by consistent execution of the test protocol for each challenge, including timing of sample collection, sampling procedures, and sample preservation.

Representativeness was also ensured by using each analytical method at its optimum capability to provide results that represent the most accurate and precise measurement it is capable of achieving.

5.8.2 Accuracy

Accuracy was quantified as the percent recovery of the parameter in a sample of known quantity. Accuracy was measured through use of both matrix spikes of a known quantity and certified standards during calibration of an instrument. The following equation was used to calculate percent recovery:

$$\text{Percent Recovery} = 100 \times [(X_{known} - X_{measured})/X_{known}]$$

where: X_{known} = known concentration of the measured parameter
$X_{measured}$ = measured concentration of parameter

Accuracy of the benchtop chlorine, pH, and turbidity meters was checked daily during the calibration procedures using certified check standards. Alkalinity and TDS were analyzed in batches. Certified QC standards and/or matrix spikes were run with each batch.

The percent recoveries of all matrix spikes and standards were within the allowable limits for all analytical methods.

5.8.3 Precision

Precision refers to the degree of mutual agreement among individual measurements and provides an estimate of random error. One sample per batch was analyzed in duplicate for the TDS measurements. One out of every ten samples for alkalinity was analyzed in duplicate. Duplicate municipal drinking water samples were analyzed for pH, total chlorine, and turbidity as part of the daily calibration process. Precision of duplicate analyses was measured by use of the following equation to calculate relative percent difference (RPD):

$$RPD = \left| \frac{S_1 - S_2}{S_1 + S_2} \right| \times 200$$

where:

S_1 = sample analysis result; and

S_2 = sample duplicate analysis result.

All RPDs were within NSF's established allowable limits for each parameter. Please note that samples from this evaluation for alkalinity and TDS were batched with other non-ETV samples. The duplicate analysis requirements apply to the whole batch, not just the samples from this ETV.

5.8.4 Completeness

Completeness is the proportion of valid, acceptable data generated using each method as compared to the requirements of the test/QA plan. The completeness objective for data generated during verification testing is based on the number of samples collected and analyzed for each parameter and/or method, as presented in Table 5-1.

Table 5-1. Completeness Requirements

Number of Samples per Parameter and/or Method	Percent Completeness
0-10	80%
11-50	90%
> 50	95%

Completeness is defined as follows for all measurements:

$$\%C = (V/T) \times 100$$

where:

%C = percent completeness;
V = number of measurements judged valid; and
T = total number of measurements.

5.8.4.1 Completeness Measurements

- All planned water chemistry samples were collected and analyzed, except for one missed pH sample during the fr virus challenge. Eleven of twelve planned pH samples were collected, for a completeness percentage of 92%.
- All scheduled *B. diminuta*, *C. parvum*, and virus samples were collected and analyzed with acceptable results.

Chapter 6
References

ASTM International (2001). D 3862-80, Standard Test Method for Retention Characteristics of 0.2-μm Membrane Filters Used in Routine Filtration Procedures for the Evaluation of Microbiological Water Quality.

ASTM International (2003). D 6908-03, Standard Practice for Integrity Testing of Water Filtration Membrane Systems.

NSF International (2004). *NSF International Laboratories Quality Assurance Manual.*

NSF International (2005a). NSF/ANSI 55-2005, *Ultraviolet microbiological water treatment systems.*

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USEPA (2004). *Water Security Research and Technical Support Action Plan*. EPA/600/R-04/063.

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Appendix A
Koch Membrane Systems Membrane Cleaning Procedure

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KOCH MEMBRANE SYSTEMS

PRE-STARTUP CLEANING PROCEDURE FOR HOLLOW FIBER PMPW AND TARGA® CARTRIDGES

The following cleaning procedure should be performed prior to initial use of cartridges. This procedure will remove preservative and condition membranes for production. Failure to follow this recommendation may lead to poor performance.

Step 1. Rinse — Ambient Temp — 10 min.

Fill system with clean water (50-85°F/10-30°C). Water should have less than 60 mg/liter $CaCO_3$ hardness.

Circulate water at standard pressure and flow conditions for 10 minutes, with permeate valve open and permeate returning to CIP tank.

Drain system and CIP tank.

Step 2. Alkaline Cycle — pH 12/Max. 110°F — 30 min.

Heat clean water (85-100°F/30-43°C) using water with less than 60 mg/liter $CaCO_3$ hardness.

Circulate heated water through system under standard pressure and flow conditions.

Add caustic slowly to achieve pH 12.0 using sodium hydroxide solution (e.g. 50% w/w).

Circulate caustic solution for 20-30 minutes.

Drain, then rinse and drain per Step 1.

Step 3. Alkaline/Chlorine Cycle — pH 12/200 ppm NaOCl — 30 min.

Heat clean water (85-110°F/30-43°C) using water with less than 60 mg/liter $CaCO_3$ hardness.

Circulate heated water through system under standard pressure and flow conditions.

Add caustic slowly to achieve pH 12.0 using sodium hydroxide solution (e.g. 50% w/w).

Add liquid sodium hypochlorite (NaOCl) to achieve 150-200 mg/liter total chlorine concentration.

Circulate caustic/chlorine solution for 20-30 minutes. Check chlorine concentration and add NaOCl as needed to maintain at 150-200 mg/liter during this time.

Drain, then rinse and drain per Step 1.

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KOCH MEMBRANE SYSTEMS™

Step 4.	Rinse	Ambient Temp	10 min.

Repeat Step 1 until pH of water in system and CIP tanks is equal to pH of incoming feed water.

NOTE: ALWAYS ADD CAUSTIC BEFORE CHLORINE. NEVER ADD CHLORINE TO A NEUTRAL OR ACID SOLUTION.

New cartridges should be kept in their original shipping containers and crates until ready for installation. Cartridges should be stored as follows:

Store cartridges indoors and out of direct sunlight.
Store cartridges at temperatures between 50-85°F/10-30°C.
Store cartridges at relative humidity below 70%.
Store cartridges in a horizontal position.

For technical assistance, please contact a Cleaning Specialist at (978) 694-7050.
To place an order, please call our Customer Service Department at (978) 694-7005.

PRE-STARTUP CLEANING PROCEDURE FOR HOLLOW FIBER PMPW AND TARGA® CARTRIDGES

Appendix B
Bacteria, Cyst, and Virus Counts, and Water Chemistry Data

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Table A-1. fr Challenge Data

Sample	Feed/Filtrate Triplicate Counts (PFU/mL)	Feed/Filtrate Geometric Mean (PFU/mL)	Log_{10} Feed/ Filtrate	Log_{10} Reduction
System Flush Sample Feed Water	<1, <1, <1			
Start-up	$6.8x10^4$, $5.9x10^4$, $5.3x10^4$	$6.0x10^4$	4.8	
15 Minute	$6.1x10^4$, $5.3x10^4$, $6.7x10^4$	$6.0x10^4$	4.8	
30 Minute	$7.9x10^4$, $8.0x10^4$, $9.0x10^4$	$8.3x10^4$	4.9	
Geometric Mean Feed		$6.7x10^4$	4.8	
Cartridge 1 Filtrate Samples				
Start-up	<1, <1, <1	<1	0.0	4.8
15 Minutes	2, 2, <1	2	0.3	4.5
30 Minutes	1, <1, <1	1	0.0	4.9
Cartridge 2 Filtrate Samples				
Start-up	<1, <1, <1	<1	0.0	4.8
15 Minutes	2, 1, <1	1	0.0	4.8
30 Minutes	2, 1, 4	2	0.3	4.6

Table A-2. MS2 Challenge Data

Sample	Feed/Filtrate Triplicate Counts (PFU/mL)	Feed/Filtrate Geometric Mean (PFU/mL)	Log_{10} Feed/ Filtrate	Log_{10} Reduction
System Flush Sample Feed Water	<1, <1, <1			
Start-up	$9.8x10^4$, $1.03x10^5$, $1.12x10^5$	$1.0x10^5$	5.0	
15 Minute	$4.2x10^4$, $5.7x10^4$, $5.4x10^4$	$5.1x10^4$	4.7	
30 Minute	$1.23x10^5$, $9.8x10^4$, $1.12x10^5$	$1.1x10^5$	5.0	
Geometric Mean Feed		$8.3x10^4$	4.9	
Cartridge 1 Filtrate Samples				
Start-up	<1, <1, <1	<1	0.0	5.0
15 Minutes	<1, <1, <1	<1	0.0	4.7
30 Minutes	1, <1, <1	1	0.0	5.0
Cartridge 2 Filtrate Samples				
Start-up	<1, <1, <1	<1	0.0	5.0
15 Minutes	<1, <1, 1	1	0.0	4.7
30 Minutes	<1, <1, 1	1	0.0	5.0

Table A-3. *B. diminuta* Challenge Data

Sample	Feed/Filtrate Triplicate Counts (CFU/100mL)	Feed/Filtrate Geometric Mean (CFU/100mL)	Log_{10} Feed/ Filtrate	Log_{10} Reduction
System Flush Sample Feed Water	<1, <1, <1			
Start-up	$7.8x10^7$, $9.2x10^7$, $7.1x10^7$	$8.0x10^7$	7.9	
15 Minute	$9.9x10^7$, $9.5x10^7$, $6.8x10^7$	$8.6x10^7$	7.9	
30 Minute	$7.5x10^7$, $8.7x10^7$, $7.6x10^7$	$7.9x10^7$	7.9	
Geometric Mean Feed		$8.2x10^7$	7.9	
Cartridge 1 Filtrate Samples				
Start-up	1, <1, <1	1	0.0	7.9
15 Minutes	1, <1, <1	1	0.0	7.9
30 Minutes	4, <1, <1	2	0.3	7.6
Cartridge 2 Filtrate Samples				
Start-up	<1, 1, <1	1	0.0	7.9
15 Minutes	<1, <1, <1	<1	0.0	7.9
30 Minutes	<1, 3, <1	1	0.0	7.9

Table A-4. Kanamycin Resistant *B. diminuta* Challenge Data

Sample	Feed/Filtrate Triplicate Counts (CFU/100mL)	Feed/Filtrate Geometric Mean (CFU/100mL)	Log_{10} Feed/ Filtrate	Log_{10} Reduction
System Flush Sample Feed Water	<1, <1, <1			
Start-up	$6.2x10^7$, $5.0x10^7$, $7.0x10^7$	$6.0x10^7$	7.8	
15 Minute	$4.5x10^7$, $4.7x10^7$, $4.3x10^7$	$4.5x10^7$	7.7	
30 Minute	$7.5x10^7$, $4.7x10^7$, $5.8x10^7$	$5.9x10^7$	7.8	
Geometric Mean Feed		$5.4x10^7$	7.7	
Cartridge 1 Filtrate Samples				
Start-up	<1, <1, <1	<1	0.0	7.8
15 Minutes	<1, <1, <1	<1	0.0	7.7
30 Minutes	<1, <1, 1	1	0.0	7.8
Cartridge 2 Filtrate Samples				
Start-up	84, 125, 131	110	2.1	5.7
15 Minutes	42, 39, 31	37	1.6	6.1
30 Minutes	35, 38, 31	35	1.5	6.3

Table A-5. Kanamycin Resistant *B. diminuta* Retest Challenge Data

Sample	Feed/Filtrate Triplicate Counts (CFU/100mL)	Feed/Filtrate Geometric Mean (CFU/100mL)	Log_{10} Feed/ Filtrate	Log_{10} Reduction
System Flush Sample	2, 2, 3			
Feed Water				
Start-up	$1.15x10^7$, $1.23x10^7$, $1.14x10^7$	$1.17x10^7$	7.07	
15 Minute	$1.24x10^7$, $1.24x10^7$, $1.17x10^7$	$1.22x10^7$	7.09	
30 Minute	$9.7x10^6$, $1.24x10^7$, $1.28x10^7$	$1.2x10^7$	7.1	
Geometric Mean Feed		$1.2x10^7$	7.1	
Cartridge 2 Filtrate Samples				
15 Minutes	<1, 1, 1	1	0.0	7.1
30 Minutes	10, 4, 6	6	0.8	6.3

Table A-6. *C. Parvum* Challenge Data

Sample	Feed/Filtrate Triplicate Counts (cysts/L)	Feed/Filtrate Geometric Mean (cysts/L)	Log_{10} Feed/ Filtrate	Log_{10} Reduction
System Flush Sample	<1, <1, <1			
Feed Water				
Start-up	$4.7x10^5$, $2.2x10^5$, $8.8x10^5$	$4.5x10^5$	5.7	
15 Minute	$9.2x10^5$, $7.5x10^5$, $3.7x10^5$	$6.3x10^5$	5.8	
30 Minute	$1.0x10^6$, $7.4x10^5$, $1.8x10^5$	$5.1x10^5$	5.7	
Geometric Mean Feed		$5.3x10^5$	5.7	
Cartridge 1 Filtrate Samples				
Start-up	<1, <1, <1	<1	0.0	5.7
15 Minutes	<1, <1, <1	<1	0.0	5.8
30 Minutes	<1, <1, <1	<1	0.0	5.7
Cartridge 2 Filtrate Samples				
Start-up	<1, <1, <1	<1	0.0	5.7
15 Minutes	<1, <1, <1	<1	0.0	5.8
30 Minutes	<1, <1, <1	<1	0.0	5.7