

# Environmental Technology Verification Report


## Removal of Microbial Contaminants in Drinking Water

Siemens Corporation  
Memcor<sup>®</sup> L20V Ultrafiltration Module

Prepared by



NSF International

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

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# THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM



U.S. Environmental Protection Agency



NSF International

## ETV Joint Verification Statement

<b>TECHNOLOGY TYPE:</b>	<b>ULTRAFILTRATION</b>
<b>APPLICATION:</b>	<b>REMOVAL OF MICROBIAL CONTAMINANTS</b>
<b>PRODUCT NAME:</b>	<b>MEMCOR® L20V ULTRAFILTRATION MODULE</b>
<b>VENDOR:</b>	<b>SIEMENS WATER TECHNOLOGIES CORPORATION</b>
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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 Siemens Memcor® L20V ultrafiltration (UF) module for removal of microbial contaminants under controlled laboratory challenge conditions. The challenge tests were conducted at NSF's testing laboratory in Ann Arbor, MI. Testing of the Siemens Memcor® L20V ultrafiltration membrane module was conducted to verify microbial reduction performance under the membrane challenge requirements of the USEPA Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR).

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 permittees), 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

The Siemens Memcor L20V UF module was tested for removal of endospores of the bacteria *Bacillus atrophaeus* and the MS2 coliphage virus according to the requirements of the EPA Long-Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR). *B. atrophaeus* served as a surrogate for *Cryptosporidium* oocysts. Five modules from five different production lots were challenged with both organisms. Separate challenges were conducted for each organism. The modules were operated at a target flux of 80 gallons per square foot per day (gfd), which for the L20V equates to approximately 22.8 gallons per minute (gpm).

The LT2ESWTR specifies that log removal values (LRV) be calculated for each module for each organism, and then one LRV for each organism (LRV<sub>C-TEST</sub>) be assigned from the set of LRV. However, the rule does not specify how the LRV<sub>C-TEST</sub> should be determined, instead, three different methods are suggested. All three methods were used to assign LRV for this verification. See the Verification of Performance section below for descriptions of each method. The LRV<sub>C-TEST</sub> for each method are presented in Table VS-i.

<b>Challenge Organism</b>	<b>Method 1</b>	<b>Method 2</b>	<b>Method 3</b>
<i>B. atrophaeus</i>	6.89	6.89	6.81
MS2	2.49	2.50	2.22

## PRODUCT DESCRIPTION

The Memcor L20V UF membrane module is a member of the Memcor XP line of products. The module measures 4.7 inches in diameter by 70.9 inches in length. The membrane fibers are made of polyvinylidene fluoride (PVDF). Water flow through the membrane fibers is outside to inside. The modules operate in a dead-end mode, with no reject stream. The nominal pore size is 0.04 µm.

Siemens supplied five modules from five different production runs for testing. The modules were tested in a pilot unit supplied by Siemens.

## VERIFICATION TEST DESCRIPTION

### *Challenge Organisms*

The L20V modules were tested for removal of microorganisms using endospores of the bacteria *Bacillus atrophaeus* (ATCC 9372, deposited as *Bacillus subtilis* var. *niger*), and MS-2 coliphage virus (ATCC 15597-B1). *B. atrophaeus* served as a surrogate for *Cryptosporidium* oocysts, as well as other bacteria. *B. atrophaeus* endospores are ellipsoidal (football shaped), with an average diameter of 0.8 µm, and an average length of 1.8 µm. A full discussion of the rationale for using *Bacillus* endospores as a surrogate for *Cryptosporidium* can be found in the verification report. Virus removal testing was conducted using MS-2 for possible virus removal credits. MS-2 is considered a suitable surrogate for pathogenic viruses because of its small size, at 24 nm in diameter. Separate challenge tests were conducted for each challenge organism, so each module was tested twice over the course of the testing activities.

### *Test Site and Challenge Water*

The microbial challenge tests were conducted at NSF's testing laboratory in Ann Arbor, MI. Local tap water was treated by carbon filtration, reverse osmosis, ultraviolet disinfection, and deionization to make the base water for the tests. A water supply tank was filled with the base water, and sodium bicarbonate

was added in sufficient quantity to provide alkalinity at a target of  $100 \pm 10$  mg/L as calcium carbonate. The pH was then lowered with hydrochloric acid to a target range of  $7.5 \pm 0.5$ .

### ***Methods and Procedures***

The tests followed the procedures described in the Test/QA Plan for the Microbial Seeding Challenge Study of the Siemens Memcor L10V, L20V, and S10V Ultrafiltration Modules. The challenge protocol was adapted from the ETV Protocol for Equipment Verification Testing for Physical Removal of Microbiological and Particulate Contaminants, and the USEPA Membrane Filtration Guidance Manual (MFGM).

The pilot unit holds three modules, but each module was tested separately. Each module was tested in the same housing. The other two housings were closed off. The target flux for the tests was 80 gallons per square foot per day (gfd), which equals a flow rate of 22.8 gallons per minute (gpm) for the L20V module.

Before and after each challenge test, the modules were subjected to a two minute pressure decay test using the program in the pilot unit's programmable logic controller (PLC). Siemens defined a passing pressure decay test as less than or equal to 1.5 psi per minute. The PLC gives a warning message if this decay rate is exceeded.

Prior to the start of each challenge test, the module and pilot unit were flushed for approximately two minutes, and at the end of the flush a negative control sample was collected from the filtrate sample tap. The duration of each microbial challenge test was 30 minutes. Feed and filtrate grab samples were collected for challenge organism enumeration after three minutes of operation, after 15 minutes of operation, and after 30 minutes of operation. The challenge organisms were intermittently injected into the feed stream for five-minute periods using a peristaltic pump at each sampling point. The injection point was downstream of the pilot unit's feed tank, as shown in Figure 2-1. During each injection period, the challenge organism was fed to the feed stream for at least 3 minutes prior to collection of the feed and filtrate samples during the fourth and/or fifth minutes. At the end of each challenge test, a second pressure decay test was conducted to confirm membrane integrity.

The MFGM suggests that feed and filtrate samples not be collected until at least three hold-up volumes of water containing the challenge organism have passed through the membrane to establish equilibrium (equilibrium volume). The hold-up volume is defined as the "unfiltered test solution volume that would remain in the system on the feed side of the membrane at the end of the test." Siemens has calculated that the hold-up volume for the Memcor CP pilot unit with only one membrane cartridge in place is 8 gallons, not including the unit's feed tank. The microbial challenges were conducted at approximately 22.8 gpm, so over 68 gallons of spiked feed water passed through the membranes prior to sample collection, well over the equilibrium volume.

### **VERIFICATION OF PERFORMANCE**

The LT2ESWTR and MFGM specify that an LRV for the test ( $LRV_{C-TEST}$ ) be calculated for each module tested, and that the LRV for each module are then combined to yield a single  $LRV_{C-TEST}$  for the product. If fewer than 20 modules are tested, as was the case for this verification, the  $LRV_{C-TEST}$  is simply the lowest LRV for the individual modules. However, the rule does not specify a method to calculate  $LRV_{C-TEST}$  for each module. Suggested options in the MFGM include the following: calculating a LRV for each feed/filtrate sample pair, then calculating the average of the LRV (Method 1); averaging all of the feed and filtrate counts, and then calculating a single LRV for the module (Method 2); or calculating a LRV for each feed/filtrate sample pair, and then selecting the LRV for the module as the lowest (most conservative of the three options, Method 3).

All three approaches for calculating the LRV are reported here. Note the LT2ESWTR and MFGM do not specify whether the averages should be calculated as the arithmetic mean or geometric mean. For this verification, geometric means were calculated.

All pressure decay rates were below 0.08 psig/min, indicating that there were no membrane integrity issues during the tests.

**B. atrophaeus Reduction**

The LT2ESWTR indicates a maximum challenge concentration to achieve a reduction of  $6.5 \log_{10}$  ( $3.16 \times 10^6$  CFU/100 mL). The *B. atrophaeus* feed concentrations for these tests ranged from  $6.5 \times 10^6$  to  $1.7 \times 10^7$  CFU/100 mL, taking into account the expected percent recovery of the challenge organism, which is typically less than 100%. The *B. atrophaeus* LRV from the three different calculation methods are presented in Table VS-ii. The  $LRV_{C-TEST}$  for each method is in bold font. The LT2ESWTR specifies that the maximum possible  $LRV_{C-TEST}$  awarded to a membrane product is  $6.5 \log_{10}$ , but the LRV above 6.5 are still presented here.

No *B. atrophaeus* endospores were found in any of the filtrate samples for the Modules 1 and 2, but *B. atrophaeus* at only 1 CFU/100 mL was found in some of the filtrate samples for Modules 3, 4, and 5. Endospores were also found in the flush samples for Modules 3 and 4, at 1 and 3 CFU/100 mL, respectively. Therefore, the observed filtrate counts could be due to low level contamination of the pilot unit by the challenge organism. Since all filtrate samples were either 1 or <1 CFU, the filtrate log transformations are all 0.0. This makes the LRV simply a function of the log of the challenge concentration. The flow rates measured during the *B. atrophaeus* challenges translated into fluxes ranging from 79.5 to 80.9 gfd.

Module #	Method 1	Method 2	Method 3
Module 1	<b>6.89<sup>(1)</sup></b>	<b>6.89<sup>(1)</sup></b>	<b>6.81<sup>(1)</sup></b>
Module 2	6.90	6.90	6.89
Module 3	6.91	6.91	6.90
Module 4	7.00	7.00	6.99
Module 5	7.07	7.07	6.97

(1)  $LRV_{C-TEST}$  under these two methods should be capped at 6.5.

**MS-2 Reduction**

The MS-2 feed concentrations ranged from  $2.5 \times 10^4$  PFU/mL to  $7.4 \times 10^4$  PFU/mL. The LRV for MS-2 reduction are shown in Table VS-iii. The  $LRV_{C-TEST}$  for each method is in bold font. The maximum individual filtrate count was 212 PFU/mL for Module 2 at start-up. The flow rates measured during the MS-2 challenges translated into fluxes ranging from 79.2 to 80.6 gfd.

Module #	Method 1	Method 2	Method 3
Module 1	3.26	3.28	2.84
Module 2	2.58	2.58	2.32
Module 3	<b>2.49</b>	<b>2.50</b>	<b>2.22</b>
Module 4	2.70	2.70	2.39
Module 5	2.84	2.84	2.65

**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 09/30/09

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Date

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 09/31/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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# **Environmental Technology Verification Report**

## **Removal of Microbial Contaminants in Drinking Water**

### **Siemens Corporation Memcor<sup>®</sup> L20V Ultrafiltration Module**

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

## **Notice**

*The U.S. Environmental Protection Agency, through its Office of Research and Development, funded and managed, or partially funded and collaborated in, the research described herein. It has been subjected to the Agency's peer and administrative review and has been approved for publication. Any opinions expressed in this report are those of the author(s) and do not necessarily reflect the views of the Agency, therefore, no official endorsement should be inferred. Any mention of trade names or commercial products does not constitute endorsement or recommendation for use.*

## Foreword

The EPA is charged by Congress with protecting the nation's air, water, and land 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, the EPA's Office of Research and Development provides data and science support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

The Environmental Technology Verification (ETV) Program has been established by the EPA to verify the performance characteristics of innovative environmental technology across all media and to report this objective information to permittees, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of six environmental technology centers. Information about each of these centers can be found on the internet at <http://www.epa.gov/etv>.

Under a cooperative agreement, NSF International has received EPA funding to plan, coordinate, and conduct technology verification studies for the ETV "Drinking Water Systems Center" and report the results to the community at large. The DWS Center has targeted drinking water concerns such as arsenic reduction, microbiological contaminants, particulate removal, disinfection by-products, radionuclides, and numerous chemical contaminants. Information concerning specific environmental technology areas can be found on the internet at <http://www.epa.gov/nrmrl/std/etv/verifications.html>.

## **Acknowledgments**

The authors wish to acknowledge the support of all those who helped plan and conduct the verification test, analyze the data, and prepare this report. We sincerely appreciate the involvement and support of all staff from the NSF testing laboratory who were involved with testing activities for this verification. In particular, we would like to thank Mr. Sal Aridi, P.E., laboratory manager, and Mr. Kevin Schaefer, the testing engineer for this project. The NSF Microbiology Laboratory analyzed all of the feed and filtrate samples for the tests. From this laboratory, the authors would like to thank Robert Donofrio, PhD, Director of the NSF Microbiology Laboratory, Robin Bechanko, Senior Microbiologist, and Kathy O'Malley, Microbiologist. From the NSF QA Department, the authors wish to thank Joe Terrell, Supervisor of QA and Safety, for auditing the tests and also reviewing all of the test data.

Finally, we would like to thank Patrick Cook of the Michigan Department of Environmental Quality, and Jonathan Pressman of the U.S. EPA, for their reviews of this verification report.

## Table of Contents

Verification Statement .....	VS-i
Title Page .....	i
Notice .....	ii
Foreword .....	iii
Acknowledgements .....	iv
Table of Contents .....	v
List of Tables .....	vi
List of Figures .....	vi
Abbreviations and Acronyms .....	vii
Chapter 1 Introduction .....	1
1.1 ETV Program Purpose and Operation .....	1
1.2 Purpose of Verification .....	1
1.3 Testing Participants and Responsibilities .....	2
1.3.1 NSF International .....	2
1.3.2 U.S. Environmental Protection Agency .....	2
1.3.3 Siemens Corporation .....	2
Chapter 2 Product Description .....	3
2.1 UF Membrane General Description .....	3
2.2 Memcor L20V Membrane Module Description .....	3
2.3 Pilot Unit Used for Testing .....	4
Chapter 3 Methods and Procedures .....	6
3.1 Introduction .....	6
3.2 Challenge Organisms .....	6
3.3 UF Module Integrity Tests .....	7
3.4 Test Water Composition .....	7
3.5 Challenge Test Procedure .....	7
3.6 Analytical Methods .....	8
Chapter 4 Results and Discussion .....	10
4.1 Pressure Decay Test Results .....	10
4.2 <i>B. atrophaeus</i> Challenge Tests .....	11
4.2.1 Choosing $LRV_{C-TEST}$ from the Averages of the Individual LRV Calculations .....	11
4.2.2 $LRV_{C-TEST}$ Calculated from the Mean Feed and Filtrate Counts .....	11
4.2.3 Choosing $LRV_{C-TEST}$ from the Individual Sample Point LRV Calculations .....	11
4.3 MS-2 Challenge Tests .....	12
4.3.1 Choosing $LRV_{C-TEST}$ from the Averages of the Individual LRV Calculations .....	13
4.3.2 $LRV_{C-TEST}$ Calculated from the Mean Feed and Filtrate Counts .....	13
4.3.3 Choosing $LRV_{C-TEST}$ from the Individual Sample Point LRV Calculations .....	14
4.4 Operational Data and Water Quality Data for All Challenges .....	14
Chapter 5 Quality Assurance/Quality Control .....	16
5.1 Introduction .....	16
5.2 Test Procedure QA/QC .....	16
5.3 Sample Handling .....	16
5.4 Chemistry Laboratory QA/QC .....	16

5.5	Microbiology Laboratory QA/QC .....	16
5.5.1	Growth Media Positive Controls .....	16
5.5.2	Negative Controls .....	16
5.6	Documentation .....	17
5.7	Data Review .....	17
5.8	Data Quality Indicators .....	17
5.8.1	Representativeness .....	17
5.8.2	Accuracy .....	17
5.8.3	Precision .....	18
5.8.4	Completeness .....	18
Chapter 6	References .....	20

## Appendices

Appendix A	Test/Quality Assurance Project Plan
Appendix B	<i>Bacillus</i> Endospores as a Surrogate for <i>C. parvum</i> Oocysts
Appendix C	Triplicate Challenge Organism Counts

## List of Tables

Table 2-1.	L20V Specifications .....	3
Table 2-2.	Serial Numbers of Tested Modules .....	4
Table 3-1.	Analytical Methods for Laboratory Analyses .....	9
Table 4-1.	Pressure Decay Data .....	10
Table 4-2.	<i>B. atrophaeus</i> Challenge Results .....	12
Table 4-3.	<i>B. atrophaeus</i> LRV <sub>C-TEST</sub> from Individual LRVs .....	12
Table 4-4.	MS-2 Challenge Results .....	13
Table 4-5.	MS-2 LRV <sub>C-TEST</sub> Calculated from the Mean Feed and Filtrate Counts .....	14
Table 4-6.	MS-2 LRV <sub>C-TEST</sub> from Individual LRVs .....	14
Table 4-7.	Operation Data .....	15
Table 4-8.	Water Chemistry Data .....	15
Table 5-1.	Completeness Requirements .....	18

## List of Figures

Figure 2-1.	Siemens Memcor CP pilot unit .....	4
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## Abbreviations and Acronyms

ATCC	American Type Culture Collection
°C	degrees Celsius
CFU	colony forming units
cm	centimeter
DWS	Drinking Water Systems
EPM	Electrophoretic Mobility
ETV	Environmental Technology Verification
°F	degrees Fahrenheit
ft	foot(feet)
gfd	gallons per square foot per day
gpm	gallons per minute
in	inch
kPa	kilopascals
L	liter
LRV	log removal value
LT2ESWTR	Long Term 2 Enhanced Surface Water Treatment Rule
m	meter
MFGM	Membrane Filtration Guidance Manual
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mM	millimolar
MWCO	molecular weight cutoff
NM	not measured
NSF	NSF International (formerly known as National Sanitation Foundation)
NTU	Nephelometric Turbidity Unit
ORD	Office of Research and Development
PFU	plaque forming unit
PLC	programmable logic controller
psig	pounds per square inch, gauge
PVDF	polyvinylidene fluoride
QA	quality assurance
QC	quality control
RPD	relative percent difference
SM	Standard Methods for the Examination of Water and Wastewater
TDS	total dissolved solids
TOC	total organic carbon
UF	ultrafiltration
µg	microgram
µm	microns
µS	microsiemens
USEPA	U. S. Environmental Protection Agency

## **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 permittees; 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 Siemens Memcor<sup>®</sup> L20V ultrafiltration (UF) membrane module was conducted to verify microbial reduction performance under the membrane challenge requirements of the USEPA Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR). *Please note that this verification only addresses the challenge testing requirement of the LT2ESWTR, not direct integrity testing or continuous indirect monitoring.*

Please also 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.



### **1.3 Testing Participants and Responsibilities**

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

#### **1.3.1 NSF International**

NSF is an independent, not-for-profit organization dedicated to public health and safety, and to protection of the environment. Founded in 1944 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, MI 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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#### **1.3.2 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. CR-833980-01. 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.

#### **1.3.3 Siemens Corporation**

Siemens Corporation supplied the tested membrane modules, and also a pilot testing unit in which the membranes were tested. Siemens was also responsible for providing logistical and technical support, as needed.

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Fax: +1 (719) 622-5399  
Contact: Mr. Aaron Balczewski, Director of Process Technology, Memcor Products  
Email: Aaron.Balczewski@siemens.com

## Chapter 2 Product Description

### 2.1 UF Membrane 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 can be either “inside-out” or “outside-in”. 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 microns ( $\mu\text{m}$ ). 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 Memcor L20V Membrane Module Description

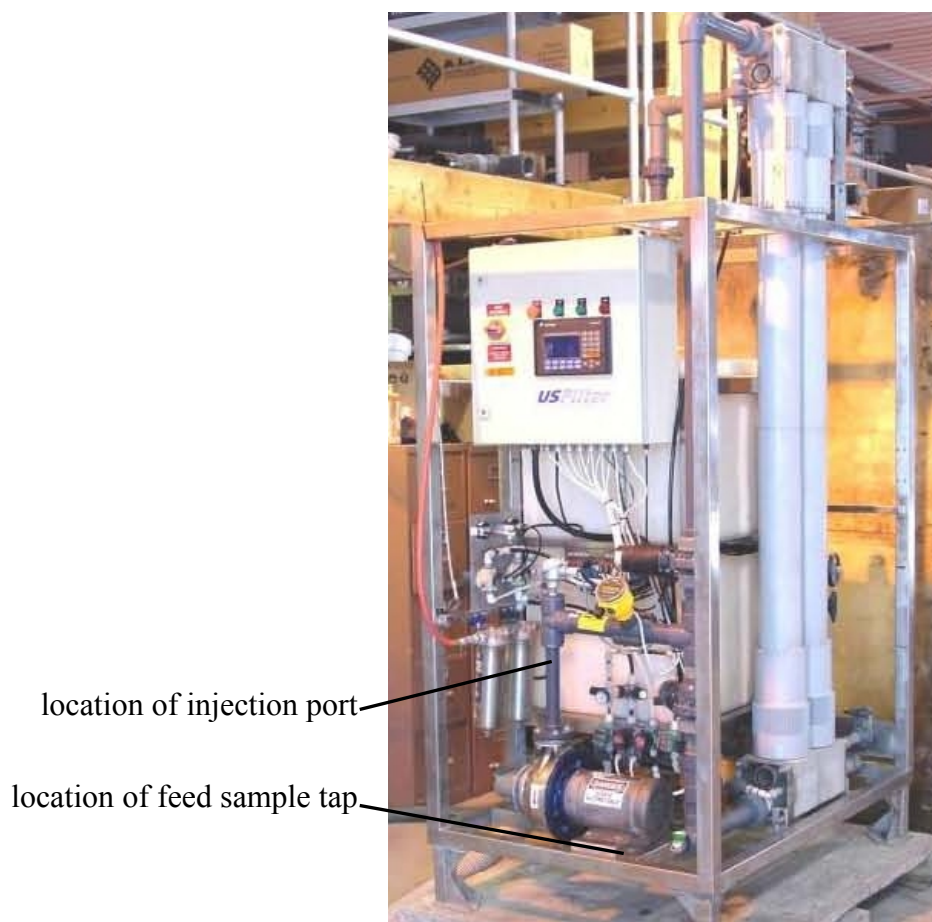
The Memcor L20V UF membrane module is a member of the Memcor CP line of products. The module measures 4.7 inches in diameter by 70.9 inches in length. The membrane fibers are made of polyvinylidene fluoride (PVDF). Water flow through the membrane fibers is outside to inside. The modules operate in a dead-end mode, with no reject stream. The module specifications are listed below in Table 2-1. The identification numbers and serial numbers for the tested modules are listed in Table 2-2. Five modules from five different production lots were submitted by Siemens for testing. The assigned module numbers in Table 2-2 correspond to the module numbers in the Results and Discussion chapter.

Parameter	Specification
Dimensions:	
Cartridge outside diameter	4.7 inches (in) (119 millimeters (mm))
Cartridge length	70.9 in (1800 mm)
Nominal membrane pore size	0.04 $\mu\text{m}$
Maximum membrane pore size	0.1 $\mu\text{m}$
Average active membrane area (outer)	410 square feet ( $\text{ft}^2$ ) (38 square meters ( $\text{m}^2$ ))
Operating Limits:	
Operating temperature range	>32 – 104 Fahrenheit ( $^{\circ}\text{F}$ ) (>0 – 40 Celcius ( $^{\circ}\text{C}$ ))
Maximum temperature	113 $^{\circ}\text{F}$ (45 $^{\circ}\text{C}$ )
Max. transmembrane pressure	21.7 pounds per square inch, gauge (psig) (150 kilopascals (kPa))
Operating pH range	2 – 10

<b>Module</b>	<b>Batch Number</b>	<b>Identification Number</b>	<b>Serial Number</b>
1	32893	WJ790145	388733
2	11207	WK7AU519	394401
3	32879	WK79K74W	388250
4	33340	WJ7AR22M	393995
5	33272	WK7AJ62G	392393

### 2.3 Pilot Unit Used for Testing

Siemens supplied a pilot unit for testing along with the membrane cartridges. A photo of a representative pilot unit is shown below as Figure 2-1. The pilot unit holds two membrane cartridges, but only one cartridge was tested at a time. The valves allowing water to pass through the other cartridge housing was closed off. The pilot unit programmable logic controller (PLC) includes an automatic pressure decay test program. This program was used to evaluate the integrity of the membranes before and after each microbial challenge test. The pilot unit automatic backflush feature was disabled for the tests.



**Figure 2-1 Siemens Memcor CP pilot unit.**

The target flow rate was entered into the PLC, and the PLC then controlled the flow through the pilot unit using internal pumps and pneumatic valves. However, the filtrate flow data presented in the results and discussion chapter was collected from an NSF installed flow meter (Great Plains Industries model A109GNA100NA10) on the filtrate line. The accuracy of the flow meter was verified prior to testing. The feed and filtrate pressure readings were taken from pressure transducers already on the pilot unit. These pressure transducers were verified by an NSF calibration officer prior to testing.

The feed water minus the challenge organism was pumped from NSF's feed tank into the pilot unit's break tank using a pump belonging to NSF. From the break tank, the pilot unit feed pump pulled water out of the break tank and sent it through the membrane module being tested. The USEPA *Membrane Filtration Guidance Manual* (EPA 815-R-06-009) calls for the challenge organism injection point to be more than ten pipe diameters upstream from the feed water sample tap. Due to the compact design of the pilot unit, the challenge organism suspension was injected using a peristaltic pump immediately upstream from the membrane feed pump, and the feed sample tap was installed immediately downstream of the feed pump, as shown in Figure 2-1. The distance between the two points was not measured, but it was assumed that the feed pump provided sufficient mixing of the water.

## Chapter 3 Methods and Procedures

### 3.1 Introduction

The challenge tests were conducted in July of 2008. The tests followed the procedures described in the *Test/QA Plan for the Microbial Seeding Challenge Study of the Siemens Memcor L10V, L20V, and S10V Ultrafiltration Modules*. The challenge protocol was adapted from the *ETV Protocol for Equipment Verification Testing for Physical Removal of Microbiological and Particulate Contaminants*, and the *USEPA Membrane Filtration Guidance Manual (MFGM)*. Note that the MFGM references the ETV protocol as an acceptable protocol for testing membrane products according to the USEPA requirements. The test/QA plan is included with this report as Appendix A.

### 3.2 Challenge Organisms

The L20V modules were tested for removal of microorganisms using the MS-2 coliphage virus, and endospores of the bacteria *Bacillus atrophaeus* (American Type Culture Collection, ATCC number 9372, deposited as *Bacillus subtilis* var. *niger*). *B. atrophaeus* was used as a surrogate for *Cryptosporidium parvum* and other protozoan oocysts. The strain of *B. atrophaeus* used for testing yields orange colonies with a distinctive morphology on trypticase soy agar (TSA), so it can be distinguished from wild-type endospores that could be present as contamination. *B. atrophaeus* endospores are ellipsoidal (football shaped), with an average diameter of 0.8  $\mu\text{m}$ , and an average length of 1.8  $\mu\text{m}$ . The LT2ESWTR allows the use of a surrogate for *C. parvum* for challenge testing to obtain *C. parvum* removal credits, provided the surrogate is conservative. The suitability of using *B. atrophaeus* as a surrogate for *Cryptosporidium* was demonstrated in the ETV verification testing of the Siemens L10V module. For that verification test, no *C. parvum* was detected in the filtrate samples for any of the five different modules tested, but *B. atrophaeus* was detected in the filtrate samples for three of the five modules, albeit at less than 1.0  $\log_{10}$  per 100 mL. See Appendix B for further discussion regarding the use of *Bacillus* endospores as a surrogate for *Cryptosporidium*.

Virus removal testing was conducted using MS-2 for possible virus removal credits. MS-2 is considered a suitable surrogate for pathogenic viruses because of its small size, at 24 nanometers in diameter.

The challenge organism suspensions were injected into the feed water stream at a sufficient rate to achieve the following target concentrations:

- MS-2 –  $1 \times 10^4$  to  $1 \times 10^5$  plaque forming units per milliliter (PFU/mL); and
- *B. atrophaeus* –  $1 \times 10^6$  to  $5 \times 10^6$  colony forming units (CFU) per 100mL.

The MFGM calls for the maximum challenge concentration to be 6.5  $\log_{10}$  above the organism's detection limit ( $3.16 \times 10^6$ ). The goal for the *B. atrophaeus* challenges was to be able to measure log reductions greater than six, so it was necessary to set the upper bound of the target range at

higher than  $3.16 \times 10^6$  CFU/100 mL to ensure that greater than  $1 \times 10^6$  CFU/100 mL were measured in the feed samples.

The MS-2 stock suspension was purchased from Biological Consulting Services of North Florida, Inc. *B. atrophaeus* was purchased from Presque Isle Cultures.

### **3.3 UF Module Integrity Tests**

Before and after each challenge test, each module was subjected to a two minute pressure decay test using the program in the pilot unit's PLC. Siemens defined a passing pressure decay test as less than or equal to 1.5 psig per minute (min). The PLC gives a warning message if this decay rate is exceeded.

### **3.4 Test Water Composition**

Local tap water was treated by carbon filtration, reverse osmosis, ultraviolet disinfection, and deionization to make the base water for the tests. The base water has the following quality control (QC) requirements for use in the NSF testing laboratory:

- Conductivity  $\leq 2$  microsiemens ( $\mu\text{S}$ ) per centimeter (cm) at  $25^\circ\text{C}$ ;
- Total organic carbon  $< 100$  micrograms ( $\mu\text{g}$ ) per L;
- Total chlorine  $< 0.05$  milligrams (mg) per L; and
- Heterotrophic bacteria plate count  $< 100$  CFU/mL.

Of the above 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 (QA)/QC program for test water quality.

A 4,000-gallon water supply tank was filled with the base water, and sodium bicarbonate was added in sufficient quantity to provide alkalinity at a target of  $100 \pm 10$  mg/L as calcium carbonate. The pH was then lowered with hydrochloric acid to a target range of  $7.5 \pm 0.5$ .

Feed samples were collected prior to each challenge period for analysis of total chlorine, alkalinity, pH, temperature, total dissolved solids, and turbidity. These samples were collected prior to addition of the challenge organism.

### **3.5 Challenge Test Procedure**

The pilot unit holds two modules, but each module was tested separately, as discussed above in Section 2.3. Each module was tested in the same cartridge housing. The other housing was closed off. The target flux for membrane operation was 80 gallons per square foot per day (gfd) at  $20^\circ\text{C}$ , which, for the L20V, equals a flow rate of 22.8 gallons per minute (gpm).

The modules were "brand new" when challenged. There was no seasoning period, or other period of operation prior to the tests to allow any sort of a cake layer to build up. Testing new modules represented a worse case field operation scenario.

Separate challenge tests were conducted for each challenge organism, so each module was tested twice. Each module was installed in the membrane chamber, and then the test engineer started

operation of the pilot unit and allowed the PLC to run through a start-up sequence of operations. Once normal filtration had started, the engineer started a pressure decay test. After the pressure decay test was complete, the normal filtration mode resumed. At this time, the engineer adjusted the flow rate to 22.8 gpm if necessary, and collected a flush sample from the filtrate sample tap.

The duration of each microbial challenge test was 30 minutes. Feed and filtrate grab samples were collected for challenge organism enumeration after three minutes of operation, after 15 minutes of operation, and after 30 minutes of operation. The challenge organisms were intermittently injected into the feed stream for five-minute periods using a peristaltic pump at each sampling point. The injection point was downstream of the pilot unit's feed tank, as shown in Figure 2-1. During each injection period, the challenge organism was fed to the feed stream for at least 3 minutes prior to collection of the feed and filtrate samples during the fourth and/or fifth minutes. At the end of each challenge test, a second pressure decay test was conducted to confirm membrane integrity.

The MFGM suggests that feed and filtrate samples not be collected until at least three hold-up volumes of water containing the challenge organism have passed through the membrane, to establish equilibrium (equilibrium volume). The hold-up volume is defined as the "unfiltered test solution volume that would remain in the system on the feed side of the membrane at the end of the test." Siemens has calculated that the hold-up volume for the Memcor CP pilot unit with only one membrane cartridge in place is 8 gallons, not including the unit's feed tank. The microbial challenges were conducted at approximately 22.8 gpm, so over 68 gallons of spiked feed water passed through the membranes prior to sample collection, well over the equilibrium volume.

### **3.6 Analytical Methods**

A list of laboratory analytical methods can be found in Table 3.1. All samples for MS-2 and *B. atrophaeus* and were analyzed in triplicate.

The following are the analytical instruments used for water quality measurements:

- Alkalinity – SmartChem Discrete Analyzer;
- pH – Orion EA 940 pH/ISE meter;
- Temperature – Fluke 51 II digital thermometer;
- Total Chlorine – Hach DR/2010 spectrophotometer using AccuVac vials; and
- Turbidity – Hach 2100P turbidimeter.

**Table 3-1. Analytical Methods for Laboratory Analyses**

<b>Parameter</b>	<b>Method</b>	<b>NSF Reporting Limit</b>	<b>Lab Accuracy (% Recovery)</b>	<b>Lab Precision (%RPD<sup>1</sup>)</b>	<b>Hold Time</b>
Alkalinity (total, as CaCO <sub>3</sub> )	USEPA 310.2	5 mg/L	90-110	≤ 13	14 days
pH	SM <sup>2</sup> 4500-H <sup>+</sup>	NA	NA	≤ 10	none <sup>3</sup>
Total Dissolved Solids (TDS)	SM 2540 C	5 mg/L	90-110	≤ 10	7 days
Total Chlorine	SM 4500-Cl G	0.05 mg/L	90-110	≤ 10	none <sup>3</sup>
Turbidity	SM 2130	0.1 NTU <sup>4</sup>	95-105		none <sup>3</sup>
MS-2	NSF 55 <sup>5</sup>	1 PFU/mL			30 hours
<i>B. atrophaeus</i>	SM 9218 <sup>6</sup>	1 CFU/100 mL	—	—	30 hours

(1) RPD = Relative Percent Deviation

(2) SM = Standard Methods

(3) Immediate analysis required

(4) NTU = Nephelometric Turbidity Unit

(5) Method published in NSF/ANSI Standard 55 – Ultraviolet Microbiological Water Treatment Systems. Method is similar to EPA Method 1601.

(6) TSA was substituted for nutrient agar in SM 9218 so that the challenge endospores could be distinguished from wild-type endospores. TSA gives orange colonies with a distinctive morphology.



## Chapter 4 Results and Discussion

The challenge tests were conducted from July 24, 2008 to July 30, 2008. One module was tested per day, with separate 30-minute challenge tests each for MS-2 and *B. atrophaeus*.

For presentation of the challenge organism data in this chapter, the observed triplicate counts were averaged by calculating geometric means, as suggested for microbial enumeration data in SM 9020. The mean counts were also log<sub>10</sub> transformed for the purpose of calculating log removal values (LRV). Samples with no organisms found were treated as 1 per unit volume for the purpose of calculating the means, unless all three triplicate counts were non-detect for the organism. The triplicate counts for each sample are presented in Appendix C.

The LT2ESWTR and MFGM specify that an LRV for the test (LRV<sub>C-TEST</sub>) be calculated for each module tested, and that the LRVs for each module are then combined to yield a single LRV<sub>C-TEST</sub> for the product. If fewer than 20 modules are tested, as was the case for this verification, the LRV<sub>C-TEST</sub> is simply the lowest LRV for the individual modules. However, the rule does not specify a method to calculate LRV<sub>C-TEST</sub> for each module. Suggested options in the MFGM include:

- Calculate a LRV for each feed/filtrate sample pair, calculate the average of the LRV;
- Average all of the feed and filtrate counts, and then calculate a single LRV for the module; or
- Calculate a LRV for each feed/filtrate sample pair, select the LRV for the module as the lowest (most conservative of the three options).

In this section, all three approaches will be used to calculate the LRV for each module. Note the LT2ESWTR and MFGM do not specify whether the averages should be calculated as the arithmetic mean or geometric mean. Since the triplicate counts were averaged by calculating geometric means, so too do the LRV calculations use geometric mean.

### 4.1 Pressure Decay Test Results

The pre-test and post-test pressure decay test results are presented in Table 4-1. All pressure decay rates were below 0.08 psig/min, indicating that there were no membrane integrity issues during the tests.

<b>Table 4-1. Pressure Decay Data</b>				
<b>Module #</b>	<b>MS-2 Pressure Decay Data (psig/min)</b>		<b><i>B. atrophaeus</i> Pressure Decay Data (psig/min)</b>	
	<b>Pre-Test</b>	<b>Post-Test</b>	<b>Pre-Test</b>	<b>Post-Test</b>
Module 1	0.07	0.06	0.06	0.06
Module 2	0.06	0.07	0.07	0.06
Module 3	0.08	0.06	0.06	0.07
Module 4	0.07	0.06	0.06	0.06
Module 5	0.07	0.07	0.07	0.07

## 4.2 *B. atrophaeus* Challenge Tests

The *B. atrophaeus* challenge results are displayed in Table 4-2. All mean feed counts exceeded the target concentration of  $1 \times 10^6$  CFU/100 mL. The LT2ESWTR indicates a maximum challenge concentration to achieve a reduction of  $6.5 \log_{10}$  ( $3.16 \times 10^6$  CFU/100 mL), so that the maximum  $LRV_{C-TEST}$  awarded to a membrane product is  $6.5 \log_{10}$ . The *B. atrophaeus* feed concentrations for these tests ranged from  $6.5 \times 10^6$  to  $1.7 \times 10^7$  CFU/100 mL. This takes into account the expected percent recovery of the challenge organism, which is typically less than 100%.

No *B. atrophaeus* was found any of the filtrate samples for Modules 1 and 2, but one endospore was found in the Module 3 30-minute sample, and one endospore per sample was found for all of the Module 4 and 5 filtrate samples. Endospores were also found in the flush samples for Modules 3 and 4, at 1 and 3 CFU/100 mL, respectively. The modules were tested in order from 1 to 5, so the fact that all of the Module 1 and 2 filtrate samples were clean, but not those for Modules 3, 4, and 5 suggests that perhaps the pilot unit became contaminated during the module change-outs. Even though endospores were detected in the filtrate samples, the counts of only 1 CFU/100mL give  $\log_{10}$  transformations of 0.0. This makes the LRV values a function of the feed concentration. All LRV are above 6.5, no matter which of the calculation methods is used. The LT2ESWTR specifies that the maximum possible  $LRV_{C-TEST}$  awarded to a membrane product is  $6.5 \log_{10}$ , but the LRV above 6.5 are still presented here.

### 4.2.1 Choosing $LRV_{C-TEST}$ from the Averages of the Individual LRV Calculations

In Table 4-2, the LRV numbers in the “Overall Mean” rows are the geometric mean calculations of the individual sample point LRVs for each module. Using this approach, the lowest LRV, and thus the  $LRV_{C-TEST}$ , is 6.89 for Module 1.

### 4.2.2 $LRV_{C-TEST}$ Calculated from the Mean Feed and Filtrate Counts

Using this approach, log values need to be calculated for each overall mean feed and filtrate count. For the *B. atrophaeus* tests, all of the log transformations of the mean feed and filtrate counts (data not shown) equaled the overall mean log values shown in Table 4-2. Therefore,  $LRV_{C-TEST}$  under this approach is also 6.89, from Module 1.

### 4.2.3 Choosing $LRV_{C-TEST}$ from the Individual Sample Point LRV Calculations

In Table 4-2, the LRV for the feed and filtrate pair at each sample point are given in the last column of the table. The lowest individual LRV for each module are listed in Table 4-3. Under this approach, the  $LRV_{C-TEST}$  is 6.81, from Module 1.

<b>Table 4-2. <i>B. atrophaeus</i> Challenge Results</b>						
	<b>Sample Point</b>	<b>Feed</b>		<b>Filtrate</b>		<b>LRV</b>
		<b>Geometric Mean (CFU/100 mL)</b>	<b>Log<sub>10</sub></b>	<b>Geometric Mean (CFU/100 mL)</b>	<b>Log<sub>10</sub></b>	
Module 1	Flush	—	—	<1	—	—
	3 Minutes	6.5x10 <sup>6</sup>	6.81	<1	0.0	6.81
	15 Minutes	8.1x10 <sup>6</sup>	6.91	<1	0.0	6.91
	30 Minutes	8.9x10 <sup>6</sup>	6.95	<1	0.0	6.95
	Overall Mean	7.8x10 <sup>6</sup>	6.89	<1	0.0	6.89
Module 2	Flush	—	—	<1	—	—
	3 Minutes	7.8x10 <sup>6</sup>	6.89	<1	0.0	6.89
	15 Minutes	7.7x10 <sup>6</sup>	6.89	<1	0.0	6.89
	30 Minutes	8.4x10 <sup>6</sup>	6.92	<1	0.0	6.92
	Overall Mean	8.0x10 <sup>6</sup>	6.90	<1	0.0	6.90
Module 3	Flush	—	—	1	—	—
	3 Minutes	8.4x10 <sup>6</sup>	6.92	<1	0.0	6.92
	15 Minutes	8.1x10 <sup>6</sup>	6.91	<1	0.0	6.91
	30 Minutes	7.9x10 <sup>6</sup>	6.90	1	0.0	6.90
	Overall Mean	8.1x10 <sup>6</sup>	6.91	1	0.0	6.91
Module 4	Flush	—	—	3	—	—
	3 Minutes	1.0x10 <sup>7</sup>	7.00	1	0.0	7.00
	15 Minutes	1.0x10 <sup>7</sup>	7.00	1	0.0	7.00
	30 Minutes	9.7x10 <sup>6</sup>	6.99	1	0.0	6.99
	Overall Mean	9.9x10 <sup>6</sup>	7.00	1	0.0	7.00
Module 5	Flush	—	—	<1	—	—
	3 Minutes	1.7x10 <sup>7</sup>	7.23	1	0.0	7.23
	15 Minutes	1.0x10 <sup>7</sup>	7.00	1	0.0	7.00
	30 Minutes	9.4x10 <sup>6</sup>	6.97	1	0.0	6.97
	Overall Mean	1.2x10 <sup>7</sup>	7.07	1	0.0	7.07

<b>Table 4-3. <i>B. atrophaeus</i> LRV<sub>C-TEST</sub> from Individual LRVs</b>	
<b>Module Number</b>	<b>LRV<sub>C-TEST</sub></b>
Module 1	6.81
Module 2	6.89
Module 3	6.90
Module 4	6.99
Module 5	6.97

### 4.3 MS-2 Challenge Tests

Table 4-4 presents the MS-2 challenge data. All mean feed counts exceeded the target of 1x10<sup>4</sup> PFU/mL. The maximum observed overall mean filtrate count was 140 PFU/mL for Module 2. The highest individual filtrate count was 212 PFU/mL for the Module 2 3-minute sample.

<b>Table 4-4. MS-2 Challenge Results</b>						
	<b>Sample Point</b>	<b>Feed</b>		<b>Filtrate</b>		<b>LRV</b>
		<b>Geometric Mean (PFU/mL)</b>	<b>Log<sub>10</sub></b>	<b>Geometric Mean (PFU/mL)</b>	<b>Log<sub>10</sub></b>	
Module 1	Flush	—	—	<1	—	—
	3 Minutes	3.8x10 <sup>4</sup>	4.58	19	1.28	3.30
	15 Minutes	4.4x10 <sup>4</sup>	4.64	9	0.95	3.69
	30 Minutes	2.9x10 <sup>4</sup>	4.46	42	1.62	2.84
	Overall Mean	3.6x10 <sup>4</sup>	4.56	19	1.25	3.26
Module 2	Flush	—	—	<1	—	—
	3 Minutes	4.5x10 <sup>4</sup>	4.65	212	2.33	2.32
	15 Minutes	6.3x10 <sup>4</sup>	4.80	136	2.13	2.67
	30 Minutes	5.6x10 <sup>4</sup>	4.75	95	1.98	2.77
	Overall Mean	5.4x10 <sup>4</sup>	4.73	140	2.14	2.58
Module 3	Flush	—	—	<1	—	—
	3 Minutes	3.3x10 <sup>4</sup>	4.52	200	2.30	2.22
	15 Minutes	4.1x10 <sup>4</sup>	4.61	127	2.10	2.51
	30 Minutes	5.0x10 <sup>4</sup>	4.70	84	1.92	2.78
	Overall Mean	4.1x10 <sup>4</sup>	4.61	129	2.10	2.49
Module 4	Flush	—	—	<1	—	—
	3 Minutes	2.5x10 <sup>4</sup>	4.40	103	2.01	2.39
	15 Minutes	3.3x10 <sup>4</sup>	4.52	47	1.67	2.85
	30 Minutes	3.7x10 <sup>4</sup>	4.57	48	1.68	2.89
	Overall Mean	3.1x10 <sup>4</sup>	4.50	61	1.78	2.70
Module 5	Flush	—	—	<1	—	—
	3 Minutes	5.4x10 <sup>4</sup>	4.73	121	2.08	2.65
	15 Minutes	5.5x10 <sup>4</sup>	4.74	84	1.93	2.81
	30 Minutes	7.4x10 <sup>4</sup>	4.87	64	1.81	3.06
	Overall Mean	6.0x10 <sup>4</sup>	4.78	87	1.94	2.84

#### 4.3.1 Choosing LRV<sub>C-TEST</sub> from the Averages of the Individual LRV Calculations

In Table 4-4, the LRV numbers in the “Overall Mean” rows are the geometric mean calculations of the individual sample point LRVs for each module. Using this approach, the lowest LRV, and thus the LRV<sub>C-TEST</sub>, is 2.49 for Module 3.

#### 4.3.2 LRV<sub>C-TEST</sub> Calculated from the Mean Feed and Filtrate Counts

Using this approach, each overall mean feed and filtrate count needs to be log transformed. In most cases these log values will be equal to the overall mean log values presented in Table 4-4 as the mean of the individual log<sub>10</sub> values. However, in some instances, the log of the overall mean feed or filtrate count will differ slightly from that calculated from the individual log values. The log transformations of the overall mean feed and filtrate counts are presented in Table 4-5. Under this approach, the LRVs for Modules 1 and 3 differ from the approach in Section 4.3.1. Module 3 still gives the LRV<sub>C-TEST</sub>, at 2.50.

Module Number	Feed		Filtrate		LRV
	Geometric Mean (PFU/mL)	Log <sub>10</sub>	Geometric Mean (PFU/mL)	Log <sub>10</sub>	
Module 1	3.6x10 <sup>4</sup>	4.56	19	1.28	3.28
Module 2	5.4x10 <sup>4</sup>	4.73	140	2.15	2.58
Module 3	4.1x10 <sup>4</sup>	4.61	129	2.11	2.50
Module 4	3.1x10 <sup>4</sup>	4.49	61	1.79	2.70
Module 5	6.0x10 <sup>4</sup>	4.78	87	1.94	2.84

### 4.3.3 Choosing LRV<sub>C-TEST</sub> from the Individual Sample Point LRV Calculations

In Table 4-4, the LRVs for the feed and filtrate pair at each sample point are given in the last column of the table. The lowest individual LRVs for each module are listed in Table 4-6. Under this approach, the LRV<sub>C-TEST</sub> is 2.22, from Module 3.

Module Number	LRV <sub>C-TEST</sub>
Module 1	2.84
Module 2	2.32
Module 3	2.22
Module 4	2.39
Module 5	2.65

### 4.4 Operational Data and Water Quality Data for All Challenges

The pilot unit operational data is presented in Table 4-7. The filtrate flow rate recordings ranged from 22.56 to 23.02 gpm. Note that the flow rate was controlled using the pilot unit's PLC, which was receiving flow rate data from the internal flow meter. However, the flow rate measurements were taken on the filtrate line outside the pilot unit using a laboratory flow meter that had been calibrated prior to the start of testing. The feed and filtrate pressure measurements were made using the existing pressure transducers on the pilot unit. Siemens' maximum trans-membrane pressure of 21.7 psig was not exceeded at any time during testing.

The water chemistry data is displayed in Table 4-8. Of the water quality parameters reported, only alkalinity and pH had target ranges specified in the test/QA plan. All alkalinity measurements were within the target range of 100 ± 10 mg/L. Two of the pH measurements were slightly above the maximum target pH of 8.0.

<b>Table 4-7. Operation Data</b>									
<b>Module #</b>	<b>Date</b>	<b>Filtrate Flow Rate (gpm)</b>		<b>Flux (gfd)</b>		<b>Feed Pressure (psig)</b>		<b>Filtrate Pressure (psig)</b>	
		<b>0 Min.</b>	<b>30 Min.</b>	<b>0 Min</b>	<b>30 Min</b>	<b>0 Min.</b>	<b>30 Min.</b>	<b>0 Min.</b>	<b>30 Min.</b>
<b><i>B. atrophaeus</i> Challenges</b>									
Module 1	07/24/08	22.89	23.02	80.4	80.9	36.8	41.4	26.6	30.0
Module 2	07/25/08	22.64	22.87	79.5	80.3	37.9	39.4	28.5	29.6
Module 3	07/28/08	22.77	22.94	80.0	80.6	43.4	49.0	33.7	38.2
Module 4	07/29/08	22.71	22.87	79.8	80.3	35.2	35.5	24.7	24.8
Module 5	07/30/08	22.86	22.96	80.3	80.6	35.2	35.9	24.6	24.9
<b>MS-2 Challenges</b>									
Module 1	07/24/08	22.62	22.86	79.4	80.3	39.9	40.9	29.1	29.7
Module 2	07/25/08	22.86	22.86	80.3	80.3	36.4	39.2	27.7	29.6
Module 3	07/28/08	22.56	22.89	79.2	80.4	40.9	48.3	31.8	37.9
Module 4	07/29/08	22.96	22.92	80.6	80.5	35.9	35.8	25.2	25.0
Module 5	07/30/08	22.87	22.94	80.3	80.6	35.1	35.7	24.6	24.8

<b>Table 4-8. Water Chemistry Data</b>							
<b>Module #</b>	<b>Date</b>	<b>Alkalinity (mg/L CaCO<sub>3</sub>)</b>	<b>pH</b>	<b>Temp. (°C)</b>	<b>Total Chlorine (mg/L)</b>	<b>TDS (mg/L)</b>	<b>Turbidity (NTU)</b>
<b><i>B. atrophaeus</i> Challenges</b>							
Module 1	07/24/08	100	8.04	24.3	<0.05	100	0.27
Module 2	07/25/08	100	7.83	25.0	<0.05	110	0.15
Module 3	07/28/08	94	7.79	23.2	<0.05	110	0.19
Module 4	07/29/08	89	7.83	24.9	<0.05	110	0.27
Module 5	07/30/08	88	7.82	24.3	<0.05	100	0.20
<b>MS-2 Challenges</b>							
Module 1	07/24/08	100	7.99	24.1	<0.05	110	0.25
Module 2	07/25/08	98	8.06	25.1	<0.05	110	0.24
Module 3	07/28/08	93	7.84	23.1	<0.05	110	0.18
Module 4	07/29/08	93	7.80	24.9	<0.05	110	0.30
Module 5	07/30/08	91	7.86	24.3	<0.05	110	0.26

## **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.

#### **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 (Appendix A). NSF QA Department staff performed an audit during testing to ensure the proper procedures were followed. The audit yielded no significant findings.

#### **5.3 Sample Handling**

All samples analyzed by the NSF Chemistry and Microbiology Laboratories were labeled with unique identification numbers. All samples were analyzed within allowable holding times.

#### **5.4 Chemistry Laboratory 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.

The NSF QA/QC requirements are all compliant with those given in the USEPA method or Standard Method for the parameter. Also, every analytical method has an NSF standard operating procedure.

#### **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.

##### **5.5.2 Negative Controls**

For each sample batch processed, an unused membrane filter and a blank with 100 mL of buffered, sterilized dilution water was 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 Microsoft™ Excel® spreadsheets. These spreadsheets were used to calculate the geometric means and log<sub>10</sub> reductions. 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. As required in the ETV Quality Management Plan, NSF ETV staff checked at least 10% 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 membrane modules were tested in a Siemens pilot unit, at the flux specified by Siemens.

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:

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

where:  $X_{\text{known}}$  = known concentration of the measured parameter  
 $X_{\text{measured}}$  = 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. At least 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 RPD:

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

where:

$S_1$  = sample analysis result; and

$S_2$  = sample duplicate analysis result.

All RPD 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.

<b>Table 5-1. Completeness Requirements</b>	
<b>Number of Samples per Parameter and/or Method</b>	<b>Percent Completeness</b>
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.

All planned samples were collected for challenge organism and water chemistry analysis, and all planned operational data readings were collected. This gives a completeness of 100% for all parameters.

## Chapter 6 References

APHA, AWWA, and WEF (2005). *Standard Methods for the Examination of Water and Wastewater*, 21st Edition.

NSF International (2007). *NSF/ANSI Standard 55 – Ultraviolet Microbiological Water Treatment Systems*.

USEPA (2005). *Membrane Filtration Guidance Manual* (EPA 815-R-06-009).

USEPA and NSF International (2005). *ETV Protocol for Equipment Verification Testing for Physical Removal of Microbiological and Particulate Contaminants*

**Appendix A**  
**Test/Quality Assurance Project Plan**

Contact Mr. Bruce Bartley at 734-769-5148 or [bartley@nsf.org](mailto:bartley@nsf.org) for a copy of this document.

## **Appendix B**

### ***Bacillus* Endospores as a Surrogate for *C. parvum* Oocysts**

The EPA LT2ESWTR allows the use of a surrogate for *C. parvum*, provided the surrogate is conservative. The EPA Membrane Filtration Guidance Manual (MFGM) specifically discusses *Bacillus subtilis* as a surrogate, but states “Because there is limited data currently available regarding the use of *Bacillus subtilis* in membrane challenge studies, a characterization of this organism would be necessary to determine whether it could be used as a *Cryptosporidium* surrogate...” The MFGM also states “Based on the size...*Bacillus subtilis* could potentially be considered a conservative surrogate...pending a comparison of other characteristics (e.g., shape, surface charge, etc.)...”

#### **1. Organism Size and Shape**

*C. parvum* is spherical in shape, while *Bacillus* endospores are ellipsoidal in shape (football shaped). *C. parvum* has a diameter of 4-6  $\mu\text{m}$ . *Bacillus* endospores are approximately 0.8  $\mu\text{m}$  in diameter, and 1.8  $\mu\text{m}$  in length. Therefore, *Bacillus* endospores are a conservative surrogate for *C. parvum*, no matter what the orientation of the endospore is when it impacts the test membrane.

Baltus et. al. (2008) studied membrane rejection of bacteria and viruses with different length vs. diameter aspect ratios. They theorized, based on a transport model for rod-shaped particles, that rejection would improve as the aspect ratio (length vs. diameter) increased for a fixed particle volume. However, their experimental results contradicted this, with similar rejection rates for particles with a range of aspect ratios. The model assumed that particles would impact the membrane with equal frequency for all particle orientations. They theorize that instead, an end-on orientation was favored for transport of the particles in the water stream. They concluded that microorganism removal by membranes could be conservatively estimated using only the rod diameter in transport models. These findings add an additional safety factor to using *Bacillus* endospores as a surrogate for *C. parvum*.

#### **2. Electrophoretic Mobility and Isoelectric Point**

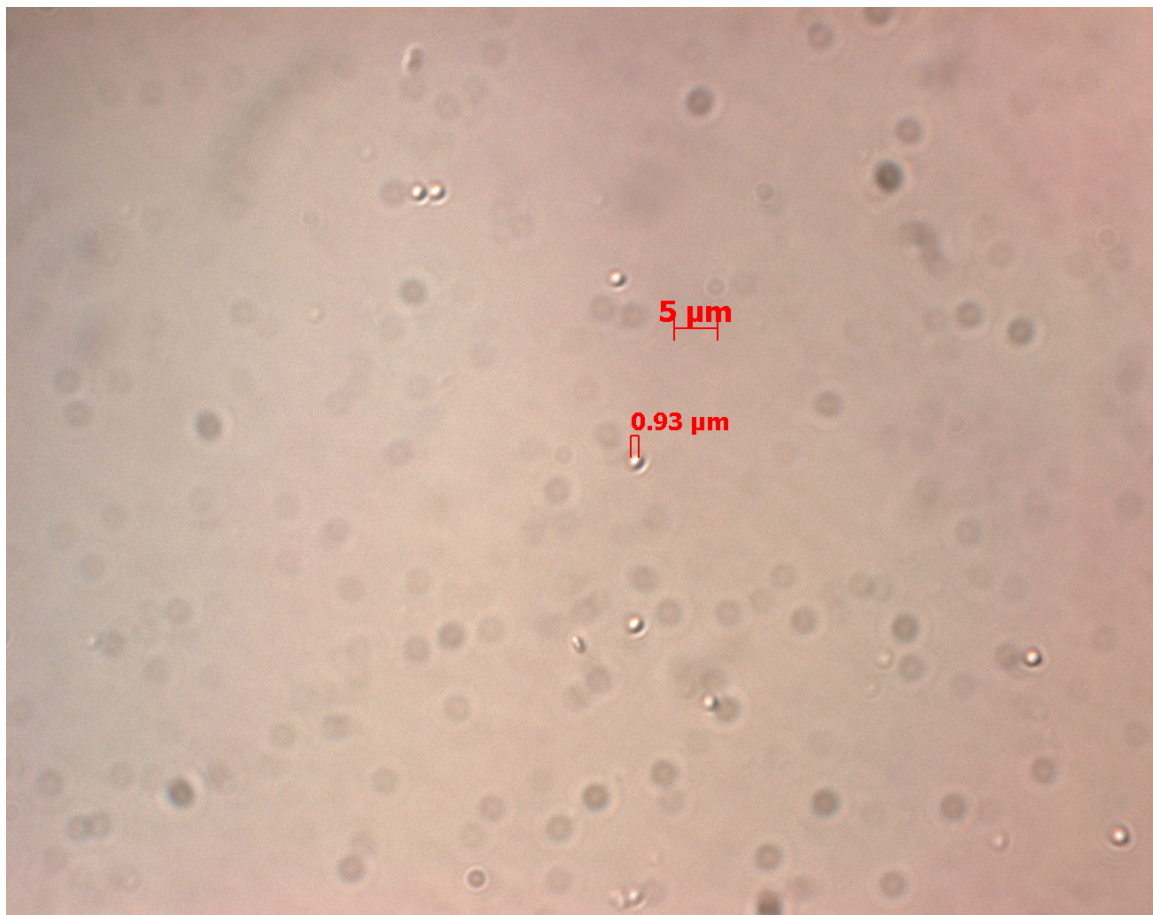
A suitable surrogate should have a surface charge similar to *C. parvum*, as measured through the isoelectric point and electrophoretic mobility (EPM). The isoelectric point is the pH at which the particle has a neutral surface charge in an aqueous environment. Below this point the particle has a net positive charge, above it a net negative charge. Many studies have pegged the isoelectric point of *C. parvum* between pH values of 2 and 4, thus it would have a negative surface charge in the neutral pH range. The isoelectric point can be found by measuring the EPM of the particle at various pH values. The pH where the EPM is zero is classified as the isoelectric point.

Lytle et. al. (2002) measured the EPM of both *C. parvum* and *B. subtilis* endospores in solutions of increasing buffer concentration (0.915 millimolar, mM, 9.15 mM, and 91.5 mM  $\text{KH}_2\text{PO}_4$ ). They found that increasing the buffer concentration also increases the EPM toward a positive

value. The buffer concentration of the test water for the Siemens tests was approximately 1 mM. Therefore, the 0.915 mM data from this study should be the most accurate representation of the *C. parvum* and *B. subtilis* EPM for the ETV tests. In 0.915 mM solutions at pH values between 7 and 8, they observed EPM of approximately  $-2.2$  to  $-2.6 \mu\text{m cm V}^{-1} \text{s}^{-1}$  for *C. parvum*, and  $-1.9$  to  $-2.2 \mu\text{m cm V}^{-1} \text{s}^{-1}$  for *B. subtilis*. For *B. subtilis*, the researchers did not measure an isoelectric point at any buffer concentration. For *C. parvum*, they did find an isoelectric point at a pH around 2.5, but only for the 9.15 mM solution. For both organisms, the 0.915 mM solution generally gave lower (more negative) EPM values than the solutions with higher buffering capacity.

### 3. Aggregation

The NSF Microbiology Laboratory microscopically examined a sample of the *B. atrophaeus* stock solutions purchased for the tests. The sample was suspended in sterile, buffered, deionized water and stirred at moderate speed for 15 minutes. The estimated cell density was  $1 \times 10^9$  CFU/100 mL, which is approximately 100 times higher than the suspensions injected into the pilot units to challenge the UF membranes. Figure 1 is a photograph of the *B. atrophaeus* endospores in the sample. The magnification is 1000x oil immersion with differential interference contrast microscopy. No evidence of endospore aggregation was found.



**Figure B-1. Mono-dispersed *B. atrophaeus* endospores used for challenge tests.**

#### 4. ETV Test Data Comparing *Bacillus* endospores and *C. parvum*

During the Siemens L10V verification test, the membranes were challenged with both *B. atrophaeus* and *C. parvum* so that removal of both organisms could be compared. No *C. parvum* was detected in the filtrate samples for any of the five L10V modules tested, but *B. atrophaeus* was detected in the filtrate samples for three of the five modules, albeit at less than 1.0 log<sub>10</sub> per 100 mL. The test data is presented in Tables B-1 and B-2.

**Table B-1. Siemens L10V *C. parvum* Challenge Results**

Module Number	Sample Point	Feed		Filtrate	
		Geometric Mean (Oocysts/L)	Log <sub>10</sub>	Geometric Mean (Oocysts/L)	Log <sub>10</sub>
Module 1	3 Minutes	6.0x10 <sup>5</sup>	5.78	<1	0.0
	15 Minutes	7.5x10 <sup>5</sup>	5.88	<1	0.0
	30 Minutes	5.7x10 <sup>5</sup>	5.76	<1	0.0
	Overall Mean	6.4x10 <sup>5</sup>	5.81	<1	0.0
Module 2	3 Minutes	3.2x10 <sup>5</sup>	5.51	<1	0.0
	15 Minutes	5.8x10 <sup>5</sup>	5.76	<1	0.0
	30 Minutes	5.8x10 <sup>5</sup>	5.76	<1	0.0
	Overall Mean	4.8x10 <sup>5</sup>	5.68	<1	0.0
Module 3	3 Minutes	4.8x10 <sup>5</sup>	5.68	<1	0.0
	15 Minutes	5.8x10 <sup>5</sup>	5.76	<1	0.0
	30 Minutes	4.1x10 <sup>5</sup>	5.61	<1	0.0
	Overall Mean	4.9x10 <sup>5</sup>	5.68	<1	0.0
Module 4	3 Minutes	4.8x10 <sup>5</sup>	5.68	<1	0.0
	15 Minutes	4.7x10 <sup>5</sup>	5.67	<1	0.0
	30 Minutes	4.7x10 <sup>5</sup>	5.67	<1	0.0
	Overall Mean	4.7x10 <sup>5</sup>	5.67	<1	0.0
Module 5	3 Minutes	5.2x10 <sup>5</sup>	5.71	<1	0.0
	15 Minutes	4.7x10 <sup>5</sup>	5.67	<1	0.0
	30 Minutes	5.1x10 <sup>5</sup>	5.71	<1	0.0
	Overall Mean	5.0x10 <sup>5</sup>	5.70	<1	0.0

Table B-2. Siemens L10V <i>B. atrophaeus</i> Challenge Results					
	Sample Point	Feed		Filtrate	
		Geometric Mean (CFU/100 mL)	Log <sub>10</sub>	Geometric Mean (CFU/100 mL)	Log <sub>10</sub>
Module 1	3 Minutes	1.0x10 <sup>7</sup>	7.00	2	0.3
	15 Minutes	9.3x10 <sup>6</sup>	6.97	1	0.0
	30 Minutes	9.0x10 <sup>6</sup>	6.95	4	0.6
	Overall Mean	9.4x10 <sup>6</sup>	6.97	2	0.3
Module 2	3 Minutes	6.0x10 <sup>6</sup>	6.78	<1	0.0
	15 Minutes	7.5x10 <sup>6</sup>	6.88	3	0.5
	30 Minutes	8.1x10 <sup>6</sup>	6.91	<1	0.0
	Overall Mean	7.1x10 <sup>6</sup>	6.85	1	0.2
Module 3	3 Minutes	9.9x10 <sup>6</sup>	7.00	<1	0.0
	15 Minutes	1.0x10 <sup>7</sup>	7.00	<1	0.0
	30 Minutes	9.6x10 <sup>6</sup>	6.98	<1	0.0
	Overall Mean	9.8x10 <sup>6</sup>	6.99	<1	0.0
Module 4	3 Minutes	1.08x10 <sup>7</sup>	7.03	<1	0.0
	15 Minutes	6.2x10 <sup>6</sup>	6.79	6	0.8
	30 Minutes	9.7x10 <sup>6</sup>	6.99	2	0.3
	Overall Mean	8.7x10 <sup>6</sup>	6.94	2	0.4
Module 5	3 Minutes	6.3x10 <sup>6</sup>	6.80	<1	0.0
	15 Minutes	7.4x10 <sup>6</sup>	6.87	<1	0.0
	30 Minutes	8.0x10 <sup>6</sup>	6.90	<1	0.0
	Overall Mean	7.2x10 <sup>6</sup>	6.86	<1	0.0

## References

Baltus, R. E., A. R. Badireddy, W. Xu, and S. Chellam (2009). Analysis of Configurational Effects on Hindered Convection of Nonspherical Bacteria and Viruses across Microfiltration Membranes. *Industrial and Engineering Chemistry Research*. In press.

Brush, C. F., M. F. Walter, L. J. Anguish, and W. C. Ghiorse (1998). Influence of Pretreatment and Experimental Conditions on Electrophoretic Mobility and Hydrophobicity of *Cryptosporidium parvum* Oocysts. *Applied and Environmental Microbiology*. 64: 4439-4445.

Butkus, M. A., J. T. Bays, and M. P. Labare (2003). Influence of Surface Characteristics on the Stability of *Cryptosporidium parvum* Oocysts. *Applied and Environmental Microbiology*. 69: 3819-3825.

Lytle, D. A., C. H. Johnson, and E. W. Rice (2002). A Systematic Comparison of the Electrokinetic Properties of Environmentally Important Microorganisms in Water. *Colloids and Surfaces B: Biointerfaces*. 24: 91-101.



**Appendix C**  
**Challenge Organism Triplicate Counts**

<b>Table C-1. MS-2 Triplicate Count Data</b>							
<b>Module</b>	<b>Sample</b>	<b>Feed (PFU/mL)</b>			<b>Filtrate (PFU/mL)</b>		
		<b>Count 1</b>	<b>Count 2</b>	<b>Count 3</b>	<b>Count 1</b>	<b>Count 2</b>	<b>Count 3</b>
Module 1	Flush	—	—	—	<1	<1	<1
	3 Minutes	4.2E+04	3.8E+04	3.5E+04	15	21	21
	15 minutes	4.7E+04	5.2E+04	3.6E+04	8	5	16
	30 minutes	3.1E+04	2.6E+04	2.9E+04	39	46	42
Module 2	Flush	—	—	—	<1	<1	<1
	3 Minutes	6.9E+04	2.9E+04	4.6E+04	231	209	197
	15 minutes	8.1E+04	5.3E+04	5.7E+04	132	129	147
	30 minutes	5.3E+04	5.6E+04	6.0E+04	80	111	96
Module 3	Flush	—	—	—	<1	<1	<1
	3 Minutes	2.8E+04	4.0E+04	3.3E+04	212	180	210
	15 minutes	4.7E+04	4.0E+04	3.7E+04	96	120	177
	30 minutes	6.1E+04	5.1E+04	4.0E+04	73	66	121
Module 4	Flush	—	—	—	<1	<1	<1
	3 Minutes	2.4E+04	2.5E+04	2.6E+04	105	96	109
	15 minutes	4.0E+04	2.7E+04	3.4E+04	43	34	69
	30 minutes	3.0E+04	4.8E+04	3.5E+04	38	74	40
Module 5	Flush	—	—	—	<1	<1	<1
	3 Minutes	6.3E+04	4.4E+04	5.8E+04	116	104	148
	15 minutes	6.6E+04	5.1E+04	4.9E+04	65	79	117
	30 minutes	6.9E+04	7.8E+04	7.4E+04	52	66	77

<b>Table C-2. <i>B. atrophaeus</i> Triplicate Count Data</b>							
<b>Module</b>	<b>Sample</b>	<b>Feed (CFU/100mL)</b>			<b>Filtrate (CFU/100mL)</b>		
		<b>Count 1</b>	<b>Count 2</b>	<b>Count 3</b>	<b>Count 1</b>	<b>Count 2</b>	<b>Count 3</b>
Module 1	Flush	—	—	—	<1	<1	<1
	3 Minutes	6.9E+06	6.6E+06	6.1E+06	<1	<1	<1
	15 minutes	7.6E+06	LA	8.7E+06	<1	<1	<1
	30 minutes	9.8E+06	LA	8.1E+06	<1	<1	<1
Module 2	Flush	—	—	—	<1	<1	<1
	3 Minutes	7.8E+06	9.0E+06	6.7E+06	<1	<1	<1
	15 minutes	6.8E+06	7.1E+06	9.3E+06	<1	<1	<1
	30 minutes	7.6E+06	7.5E+06	1.0E+07	<1	<1	<1
Module 3	Flush	—	—	—	1	1	1
	3 Minutes	7.4E+06	8.6E+06	9.3E+06	<1	<1	<1
	15 minutes	7.5E+06	8.5E+06	8.2E+06	<1	<1	<1
	30 minutes	7.8E+06	7.1E+06	9.0E+06	1	<1	<1
Module 4	Flush	—	—	—	6	1	3
	3 Minutes	1.1E+07	1.1E+07	9.5E+06	1	<1	<1
	15 minutes	9.4E+06	9.9E+06	1.1E+07	1	2	<1
	30 minutes	1.0E+07	9.3E+06	9.6E+06	<1	1	1
Module 5	Flush	—	—	—	2	1	<1
	3 Minutes	4.7E+07	9.5E+06	1.0E+07	1	<1	<1
	15 minutes	9.7E+06	9.5E+06	1.1E+07	1	<1	<1
	30 minutes	9.8E+06	7.9E+06	1.1E+07	2	<1	<1