

Environmental Technology Verification Report

Removal of Microbial Contaminants in Drinking Water

Siemens Corporation
Memcor® S10V 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

TECHNOLOGY TYPE:	ULTRAFILTRATION
APPLICATION:	REMOVAL OF MICROBIAL CONTAMINANTS
PRODUCT NAME:	MEMCOR® S10V ULTRAFILTRATION MODULE
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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 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 S10V 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, as well as other bacteria. 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 S10V equates to approximately 16.7 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_{C-TEST}) be assigned from the set of LRV. However, the rule does not specify how the LRV_{C-TEST} 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_{C-TEST} for each method are presented in Table VS-i.

Challenge Organism	Method 1	Method 2	Method 3
<i>B. atrophaeus</i>	7.10	7.13	6.62
MS2	2.98	3.00	2.57

PRODUCT DESCRIPTION

The Memcor S10V UF membrane module is a member of the Memcor CS line of products. The Memcor CS modules are submerged membranes that operate by pulling water through the membrane from the outside to the inside of the hollow fiber using vacuum pressure. The module measures 5.2 inches in diameter by 46.7 inches in length. The membrane fibers are made of polyvinylidene fluoride (PVDF). 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 S10V 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 sequentially 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 ± 10 mg/L as calcium carbonate. The pH was then lowered with hydrochloric acid to a target range of 7.5 ± 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 four modules, but only one module was tested at a time. For the other membrane slots, Siemens provided cartridge ends with fibers that had been epoxied shut. The target flux for the tests was 80 gallons per square foot per day (gfd), which equals a flow rate of 16.7 gallons per minute (gpm) for the S10V module.

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 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 then 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 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 2-2. 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 the hold-up volume of the Memcor XP pilot unit as 40 gallons, not including the unit's feed tank. The flow rates measured during ETV testing ranged from 16.70 to 16.96 gpm, so only approximately 50 gallons of the spiked test solution passed through the membrane modules before sample collection began. This volume was less than the equilibrium volume of 120 gallons, but the MS-2 filtrate counts suggest that the membranes were being subjected to the full challenge concentration when the filtrate samples were collected. The feed samples were collected upstream from the membrane holding chamber, so they are not indicative of the challenge concentration in the membrane chamber after 3 minutes of injection. However, most of the MS-2 filtrate counts for the S10V challenges were above 1×10^3 PFU/mL. These filtrate counts were similar to those measured from the ETV challenge tests of the Siemens L10V and L20V membranes, which use the same UF fibers. So, the fact that the S10V filtrate counts were similar to those from the L10V and L20V tests indirectly indicates that the S10V cartridges were exposed to adequate MS-2 challenge concentrations after only 3 minutes of injection.

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 0.7 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 $6.5 \log_{10}$ (3.16×10^6 CFU/100 mL). The *B. atrophaeus* feed concentrations for these tests ranged from 3.6×10^7 to 6.1×10^7 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 the Module 1 filtrate samples, but they were found at low levels in the filtrate samples for the rest of the modules. The maximum observed filtrate count was 10 CFU/100 mL. The flow rates measured during the *B. atrophaeus* challenges translated into fluxes ranging from 80.3 to 81.2 gfd.

Module #	Method 1	Method 2	Method 3
Module 1	7.71	7.71	7.66
Module 2	7.39	7.36	7.09
Module 3	7.10⁽¹⁾	7.13⁽¹⁾	6.62⁽¹⁾
Module 4	7.53	7.63	7.26
Module 5	7.24	7.28	6.81

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

***MS-2* Reduction**

The MS-2 feed concentrations ranged from 1.42×10^6 PFU/mL to 9.0×10^6 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 9.3×10^3 PFU/mL for Module 1 30-minute sample. The flow rates measured during the MS-2 challenges translated into fluxes ranging from 80.2 to 81.4 gfd.

Table VS-iii. MS-2 LRV Calculations			
Module #	Method 1	Method 2	Method 3
Module 1	2.98	3.00	2.57
Module 2	3.19	3.20	3.09
Module 3	3.24	3.23	3.12
Module 4	3.16	3.17	2.94
Module 5	3.64	3.64	3.61

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.

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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 09/32/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[®] S10V Ultrafiltration Module

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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.

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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(es)
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	micrometer
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[®] S10V 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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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 (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 Memcor S10V Membrane Module Description

The Memcor S10V UF membrane module is a member of the Memcor CS line of products. The Memcor CS modules are submerged membranes that operate by pulling water through the membrane from the outside to the inside of the hollow fiber using vacuum pressure. The S10V module measures 5.2 inches in diameter by 46.7 inches in length. The membrane fibers are made of polyvinylidene fluoride (PVDF). 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.

Table 2-1. S10V Specifications	
Parameter	Specification
Dimensions:	
Cartridge outside diameter	5.2 inches (in) (131 millimeters (mm))
Cartridge length	46.7 in (1186 mm)
Nominal membrane pore size	0.04 μm
Maximum membrane pore size	0.1 μm
Average active membrane area (outer)	300 square feet (ft^2) (27.9 square meters (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	17.4 pounds per square inch, gauge (psig) (120 kilopascals (kPa))
Operating pH range	2 – 10

Table 2-2. Serial Numbers of Tested Modules		
Module	Batch Number	Identification Number
1	31685	WJ75E34H
2	29480	WK68C634
3	33092	WM7AC21B
4	33075	WM7AA64B
5	31116	WJ73122L

(1) NR: not recorded

2.3 Pilot Unit Used for Testing

Siemens supplied a pilot unit for testing along with the membrane cartridges. A photo of the pilot unit in the testing laboratory is shown as Figure 2-1. The pilot unit holds four membrane cartridges, but only one cartridge was tested at a time. For the other membrane slots, Siemens supplied cartridge ends with fibers that had been epoxied shut. 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.

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 using the "bucket and stopwatch" method. The pressure drop across the membrane was measured using a differential pressure transducer supplied by Siemens on the pilot unit. The accuracy of this sensor was verified prior to testing by an NSF calibration officer.

The feed water minus the challenge organism was pumped from NSF's feed tank into the pilot unit. The pilot unit uses a vacuum pump to draw water across the membranes. The rate of the NSF feed pump was set to be equal to that of the pilot unit vacuum pump, such that a constant water level was maintained in the membrane cartridge chamber. The challenge organism was injected into the pilot unit plumbing upstream of the membrane chamber. To allow for adequate mixing of the organism, 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. This was accomplished, and there were also two 90-degree pipe bends and a turbine flow meter in between the injection point and the sample tap/membrane chamber to provide further mixing. Figure 2-2 shows the injection point and feed sample tap on the pilot unit.



Figure 2-1 Siemens Memcor CMF-S pilot unit.

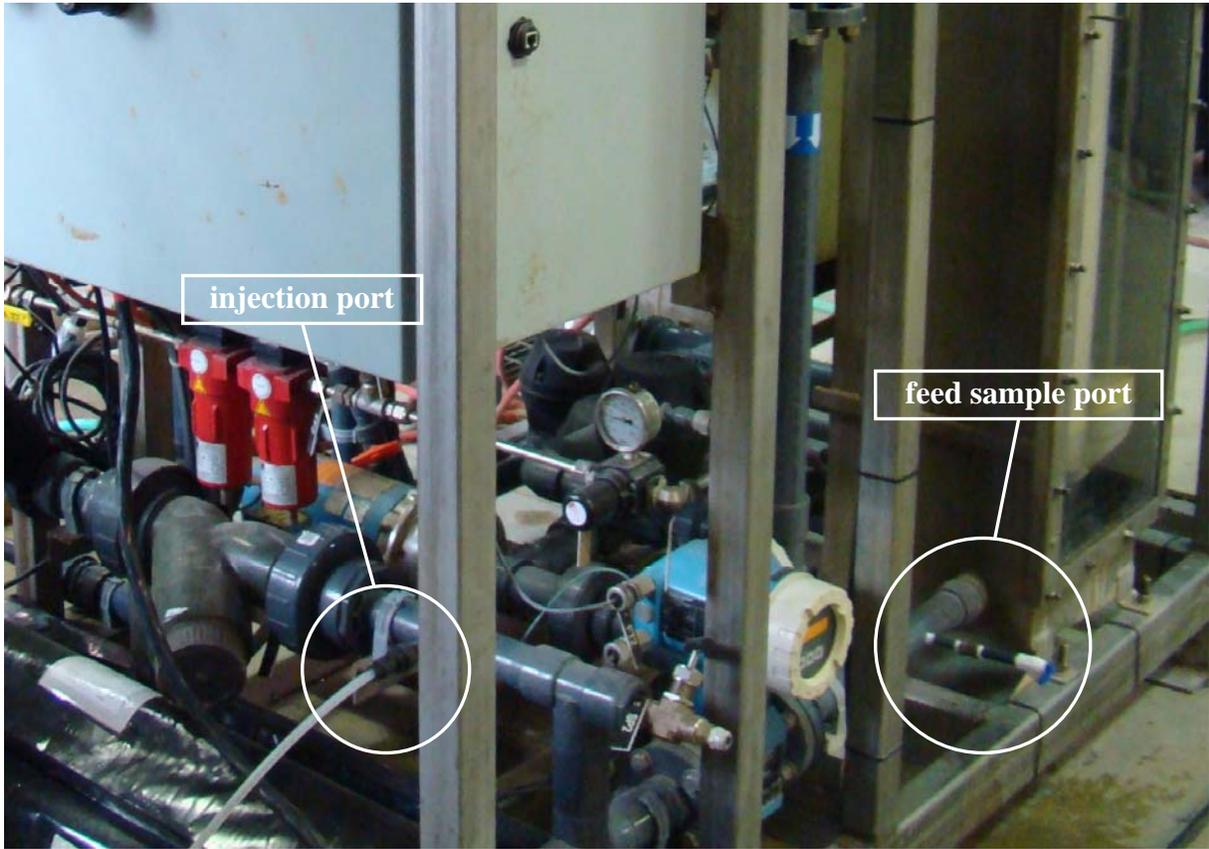


Figure 2-2. Challenge organism injection point and feed sample tap on pilot unit.

Chapter 3 Methods and Procedures

3.1 Introduction

The challenge tests were conducted in November 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 S10V 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 μm , and an average length of 1.8 μm . 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 *C. parvum* 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 a discussion of the suitability of this surrogate, and presentation of the L10V *B. atrophaeus* and *C. parvum* removal data.

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 – 1×10^4 to 1×10^5 plaque forming units per milliliter (PFU/mL); and
- *B. atrophaeus* – 1×10^6 to 5×10^6 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 (3.16×10^6). 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 3.16×10^6 CFU/100 mL to ensure that greater than 1×10^6 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. atropaeus* 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 ≤ 2 microsiemens (μS) per centimeter (cm) at 25°C ;
- Total organic carbon < 100 micrograms (μg) 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 ± 10 mg/L as calcium carbonate. The pH was then lowered with hydrochloric acid to a target range of 7.5 ± 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 four modules, but each module was tested separately, as discussed above in Section 2.3. The target flux for membrane operation was 80 gallons per square foot per day (gfd) at 20°C , which, for the L20V, equals a flow rate of 16.7 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 16.7 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 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 2-2. 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 the hold-up volume of the Memcor XP pilot unit as 40 gallons, not including the unit's feed tank. The flow rates measured during ETV testing ranged from 16.70 to 16.96 gpm, so only approximately 50 gallons of the spiked test solution passed through the membrane modules before sample collection began. This volume was less than the equilibrium volume of 120 gallons, but the MS-2 filtrate counts suggest that the membranes were being subjected to the full challenge concentration when the filtrate samples were collected. The feed samples were collected upstream from the membrane holding chamber, so they are not indicative of the challenge concentration in the membrane chamber after 3 minutes of injection. However, most of the MS-2 filtrate counts for the S10V challenges were above 1×10^3 PFU/mL. These filtrate counts were similar to those measured from the ETV challenge tests of the Siemens L10V and L20V membranes, which use the same UF fibers. So, the fact that the S10V filtrate counts were similar to those from the L10V and L20V tests indirectly indicates that the S10V cartridges were exposed to adequate MS-2 challenge concentrations after only 3 minutes of injection.

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					
Parameter	Method	NSF Reporting Limit	Lab Accuracy (% Recovery)	Lab Precision (%RPD¹)	Hold Time
Alkalinity (total, as CaCO ₃)	USEPA 310.2	5 mg/L	90-110	≤ 13	14 days
pH	SM ² 4500-H ⁺	NA	NA	≤ 10	none ³
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 ³
Turbidity	SM 2130	0.1 NTU ⁴	95-105		none ³
MS-2	NSF 55 ⁵	1 PFU/mL			30 hours
<i>B. atrophaeus</i>	SM 9218 ⁶	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 November 10, 2008 to November 14, 2008. One module was tested per day, with separate challenge tests 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_{10} 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_{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:

- 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 0.6 or 0.7 psig/min, indicating that there were no membrane integrity issues during the tests.

Table 4-1. Pressure Decay Data				
Module #	MS-2 Pressure Decay Data (psig/min)		<i>B. atrophaeus</i> Pressure Decay Data (psig/min)	
	Pre-Test	Post-Test	Pre-Test	Post-Test
Module 1	0.60	0.60	0.60	0.60
Module 2	0.70	0.70	0.70	0.70
Module 3	0.60	0.70	0.70	0.70
Module 4	0.70	0.70	0.70	0.70
Module 5	0.60	0.70	0.70	0.70

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 1×10^6 CFU/100 mL. The *B. atrophaeus* challenge results are displayed in Table 4-5. All mean feed counts exceeded the target concentration of 1×10^6 CFU/100 mL. The LT2ESWTR indicates a maximum challenge concentration to achieve a reduction of $6.5 \log_{10}$ (3.16×10^6 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 3.6×10^7 to 6.1×10^7 CFU/100 mL. This takes into account the expected percent recovery of the challenge organism, which is typically less than 100%.

B. atrophaeus endospores were found in the filtrate samples for all modules except Module 1, but at concentrations of 10 CFU/100 mL or less. One endospore was found in the flush samples for Modules 4 and 5. The modules were tested in order from 1 to 5, so the fact that an endospore was found in each of the Module 4 and 5 flush samples indicates that perhaps the pilot unit became contaminated during the module change-outs. Contamination of the pilot unit is also evident by the MS-2 flush sample counts presented later in this chapter.

Note that the Module 1 feed samples exceeded the 24-hour holding time. The samples had to be reprocessed due to laboratory error, and the reprocessing did not occur until the samples were eight days old. The Module 1 counts were in the same range as the other feed counts, indicating that the samples were not adversely affected during the holding period. This was likely because the challenge organism was an environmentally stable endospore that does not die off because it is not in a vegetative state.

For the S10V *B. atrophaeus* challenges, 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 LRV for each module. Using this approach, the lowest LRV, and thus the LRV_{C-TEST} , is 7.10 for Module 3.

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. In most cases these log values will be equal to the overall mean log values presented in Table 4-2 as the mean of the individual \log_{10} 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 4-3. Under this approach, the LRV_{C-TEST} is 7.13, from Module 3.

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 4-4. Under this approach, the LRV_{C-TEST} is 6.62, from Module 3.

	Sample Point	Feed		Filtrate		LRV
		Geometric Mean (CFU/100 mL)	Log ₁₀	Geometric Mean (CFU/100 mL)	Log ₁₀	
Module 1	Flush	—	—	<1	—	—
	3 Minutes	5.1x10 ⁷	7.71	<1	0.0	7.71
	15 Minutes	5.8x10 ⁷	7.76	<1	0.0	7.76
	30 Minutes	4.6x10 ⁷	7.66	<1	0.0	7.66
	Overall Mean	5.1x10 ⁷	7.71	<1	0.0	7.71
Module 2	Flush	—	—	<1	—	—
	3 Minutes	3.7x10 ⁷	7.57	3	0.48	7.09
	15 Minutes	4.1x10 ⁷	7.61	<1	0.0	7.61
	30 Minutes	6.1x10 ⁷	7.79	2	0.30	7.49
	Overall Mean	4.5x10 ⁷	7.66	2	0.26	7.39
Module 3	Flush	—	—	<1	—	—
	3 Minutes	4.3x10 ⁷	7.63	1	0.0	7.63
	15 Minutes	3.8x10 ⁷	7.58	3	0.48	7.10
	30 Minutes	4.2x10 ⁷	7.62	10	1.00	6.62
	Overall Mean	4.1x10 ⁷	7.61	3	0.49	7.10
Module 4	Flush	—	—	1	—	—
	3 Minutes	5.3x10 ⁷	7.72	<1	0.0	7.72
	15 Minutes	3.6x10 ⁷	7.56	2	0.30	7.26
	30 Minutes	4.2x10 ⁷	7.62	1	0.0	7.62
	Overall Mean	4.3x10 ⁷	7.63	1	0.0	7.53
Module 5	Flush	—	—	1	—	—
	3 Minutes	5.5x10 ⁷	7.74	2	0.30	7.44
	15 Minutes	5.7x10 ⁷	7.76	9	0.95	6.81
	30 Minutes	6.0x10 ⁷	7.78	2	0.30	7.48
	Overall Mean	5.7x10 ⁷	7.76	3	0.44	7.24

Module Number	Feed		Filtrate		LRV
	Geometric Mean (CFU/100 mL)	Log ₁₀	Geometric Mean (CFU/100 mL)	Log ₁₀	
Module 1	5.1x10 ⁷	7.71	<1	0.0	7.71
Module 2	4.5x10 ⁷	7.66	2	0.30	7.36
Module 3	4.1x10 ⁷	7.61	3	0.48	7.13
Module 4	4.3x10 ⁷	7.63	1	0.0	7.63
Module 5	5.7x10 ⁷	7.76	3	0.48	7.28

Module Number	LRV_{C-TEST}
Module 1	7.66
Module 2	7.09
Module 3	6.62
Module 4	7.26
Module 5	6.81

4.3 MS-2 Challenge Tests

Table 4-5 presents the MS-2 challenge data. All mean feed counts exceeded the target of 1×10^4 PFU/mL. The maximum observed overall mean filtrate count was 6.3×10^3 PFU/mL for Module 1. The highest individual filtrate count was 9.3×10^3 PFU/mL, from the Module 1 30-minute sample. Many MS-2 particles were found in the flush samples for all modules but Module 1. However, Module 1 also had the highest mean filtrate count and highest individual filtrate sample count. Therefore, the filtrate side contamination did not appear to impact the LRV_{C-TEST} for MS-2.

4.3.1 Choosing LRV_{C-TEST} from the Averages of the Individual LRV Calculations

In Table 4-5, the LRV numbers in the “Overall Mean” rows are the geometric mean calculations of the individual sample point LRV for each module. Using this approach, the lowest LRV, and thus the LRV_{C-TEST}, is 2.98 for Module 1.

4.3.2 LRV_{C-TEST} 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 4-5 as the mean of the individual \log_{10} 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 4-6. The LRV_{C-TEST} is 3.00, from Module 1.

4.3.3 Choosing LRV_{C-TEST} from the Individual Sample Point LRV Calculations

In Table 4-5, 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 4-7. Under this approach, the LRV_{C-TEST} is 2.57, from Module 1.

Table 4-5. MS-2 Challenge Results						
	Sample Point	Feed		Filtrate		LRV
		Geometric Mean (PFU/mL)	Log₁₀	Geometric Mean (PFU/mL)	Log₁₀	
Module 1	Flush	—	—	<1	—	—
	3 Minutes	7.9x10 ⁶	6.90	5.9x10 ³	3.77	3.13
	15 Minutes	9.0x10 ⁶	6.95	4.5x10 ³	3.65	3.30
	30 Minutes	3.5x10 ⁶	6.54	9.3x10 ³	3.97	2.57
	Overall Mean	6.3x10 ⁶	6.79	6.3x10 ³	3.79	2.98
Module 2	Flush	—	—	121	—	—
	3 Minutes	4.6x10 ⁶	6.66	1.84x10 ³	3.26	3.40
	15 Minutes	3.3x10 ⁶	6.52	2.7x10 ³	3.43	3.09
	30 Minutes	3.5x10 ⁶	6.54	2.8x10 ³	3.45	3.09
	Overall Mean	3.8x10 ⁶	6.57	2.4x10 ³	3.38	3.19
Module 3	Flush	—	—	317	—	—
	3 Minutes	1.65x10 ⁶	6.22	680	2.83	3.39
	15 Minutes	1.67x10 ⁶	6.22	1.26x10 ³	3.10	3.12
	30 Minutes	3.1x10 ⁶	6.49	1.87x10 ³	3.27	3.22
	Overall Mean	2.0x10 ⁶	6.31	1.2x10 ³	3.06	3.24
Module 4	Flush	—	—	335	—	—
	3 Minutes	4.4x10 ⁶	6.64	1.39x10 ³	3.14	3.50
	15 Minutes	3.5x10 ⁶	6.54	2.9x10 ³	3.46	3.08
	30 Minutes	3.6x10 ⁶	6.56	4.2x10 ³	3.62	2.94
	Overall Mean	3.8x10 ⁶	6.58	2.6x10 ³	3.40	3.16
Module 5	Flush	—	—	12	—	—
	3 Minutes	1.42x10 ⁶	6.15	340	2.53	3.62
	15 Minutes	1.50x10 ⁶	6.18	310	2.49	3.69
	30 Minutes	1.59x10 ⁶	6.20	390	2.59	3.61
	Overall Mean	1.50x10 ⁶	6.18	350	2.54	3.64

Table 4-6. MS-2 LRV_{C-TEST} Calculated from the Mean Feed and Filtrate Counts					
Module Number	Feed		Filtrate		LRV
	Geometric Mean (PFU/mL)	Log₁₀	Geometric Mean (PFU/mL)	Log₁₀	
Module 1	6.3x10 ⁶	6.80	6.3x10 ³	3.80	3.00
Module 2	3.8x10 ⁶	6.58	2.4x10 ³	3.38	3.20
Module 3	2.0x10 ⁶	6.30	1.2x10 ³	3.07	3.23
Module 4	3.8x10 ⁶	6.58	2.6x10 ³	3.41	3.17
Module 5	1.50x10 ⁶	6.18	350	2.54	3.64

Table 4-7. MS-2 LRV_{C-TEST} from Individual LRV	
Module Number	LRV_{C-TEST}
Module 1	2.57
Module 2	3.09
Module 3	3.12
Module 4	2.94
Module 5	3.61

4.4 Operational Data and Water Quality Data for All Challenges

The pilot unit operational data is presented in Table 4-8. The filtrate flow rate recordings ranged from 16.70 to 16.96 gpm. The target flow rate for the tests was 16.7 gpm, so all tests were conducted at, or slightly above, the target flow rate. 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 TMP was calculated by the pilot unit PLC using the pressure transducers on the pilot unit. These transducers were not calibrated prior to testing. Siemens' maximum trans-membrane pressure of 17.4 psig was not exceeded at any time during testing.

Table 4-8. Operation Data							
Module #	Date	Filtrate Flow Rate (gpm)		Flux (gfd)		TMP (psig)	
		0 Min.	30 Min.	0 Min	30 Min	0 Min.	30 Min.
<i>B. atrophaeus</i> Challenges							
Module 1	11/10/08	16.86	16.77	80.9	80.5	9.7	9.8
Module 2	11/11/08	16.90	16.80	81.1	80.6	8.1	8.8
Module 3	11/12/08	16.85	16.78	80.9	80.5	7.6	7.8
Module 4	11/13/08	16.86	16.91	80.9	81.2	8.8	8.9
Module 5	11/14/08	16.73	16.78	80.3	80.5	8.6	8.7
MS-2 Challenges							
Module 1	11/10/08	16.70	16.73	80.2	80.3	9.2	9.5
Module 2	11/11/08	16.91	16.78	81.2	80.5	7.7	8.4
Module 3	11/12/08	16.96	16.73	81.4	80.3	7.4	7.8
Module 4	11/13/08	16.77	16.78	80.5	80.5	8.6	8.9
Module 5	11/14/08	16.78	16.78	80.5	80.5	8.6	9.0

The water chemistry data is displayed in Table 4-9. Of the water quality parameters reported, only alkalinity and pH had target ranges specified in the test/QA plan. All alkalinity and pH measurements were within the targeted ranges.

Table 4-9. Water Chemistry Data							
Module #	Date	Alkalinity (mg/L CaCO ₃)	pH	Temp. (°C)	Total Chlorine (mg/L)	TDS (mg/L)	Turbidity (NTU)
<i>B. atrophaeus</i> Challenges							
Module 1	11/10/08	100	7.86	19.8	<0.05	130	0.44
Module 2	11/11/08	97	7.77	22.8	<0.05	120	0.49
Module 3	11/12/08	88	7.84	22.0	<0.05	100	0.47
Module 4	11/13/08	90	7.85	22.0	<0.05	100	0.42
Module 5	11/14/08	100	7.74	22.3	<0.05	110	0.20
MS-2 Challenges							
Module 1	11/10/08	100	7.79	20.0	<0.05	130	0.41
Module 2	11/11/08	98	7.78	23.2	<0.05	120	0.51
Module 3	11/12/08	95	7.73	21.9	<0.05	99	0.36
Module 4	11/13/08	90	7.75	21.5	<0.05	99	0.45
Module 5	11/14/08	94	7.70	22.3.	<0.05	110	0.25

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, except the Module 1 feed samples from the *B. atrophaeus* challenge, were analyzed within the allowable holding times. The Module 1 feed samples had to be reprocessed due to laboratory error when the samples were eight days old.

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 log₁₀ 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_{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. The NSF laboratory analyzes at least one out of every ten samples in duplicate for water chemistry parameters. However, note that since the samples from the tests described herein were analyzed in batches with other samples, the laboratory did not perform duplicate analysis on 10% of the samples from this project.

The NSF laboratory measures precision by calculating RPD using the following formula:

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

where:

S_1 = sample analysis result; and

S_2 = sample duplicate analysis result.

All RPD were within NSF's established allowable limits for each parameter.

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.

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

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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

Please contact Bruce Bartley of NSF International at bartley@nsf.org, or 734-769-5148 for a copy of the test/quality assurance project plan.

Appendix B

Bacillus* Endospores as a Surrogate for *Cryptosporidium parvum

The EPA LT2ESWTR allows the use of a surrogate for *Cryptosporidium parvum*, provided the surrogate is conservative. The EPA 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.)...” This appendix discusses supporting evidence for the use of *Bacillus* endospores as a surrogate for *C. parvum*.

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 μm . *Bacillus* endospores are approximately 0.8 μm in diameter, and 1.8 μm 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 KH_2PO_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 1×10^9 CFU/100 mL, which is more than 10 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.

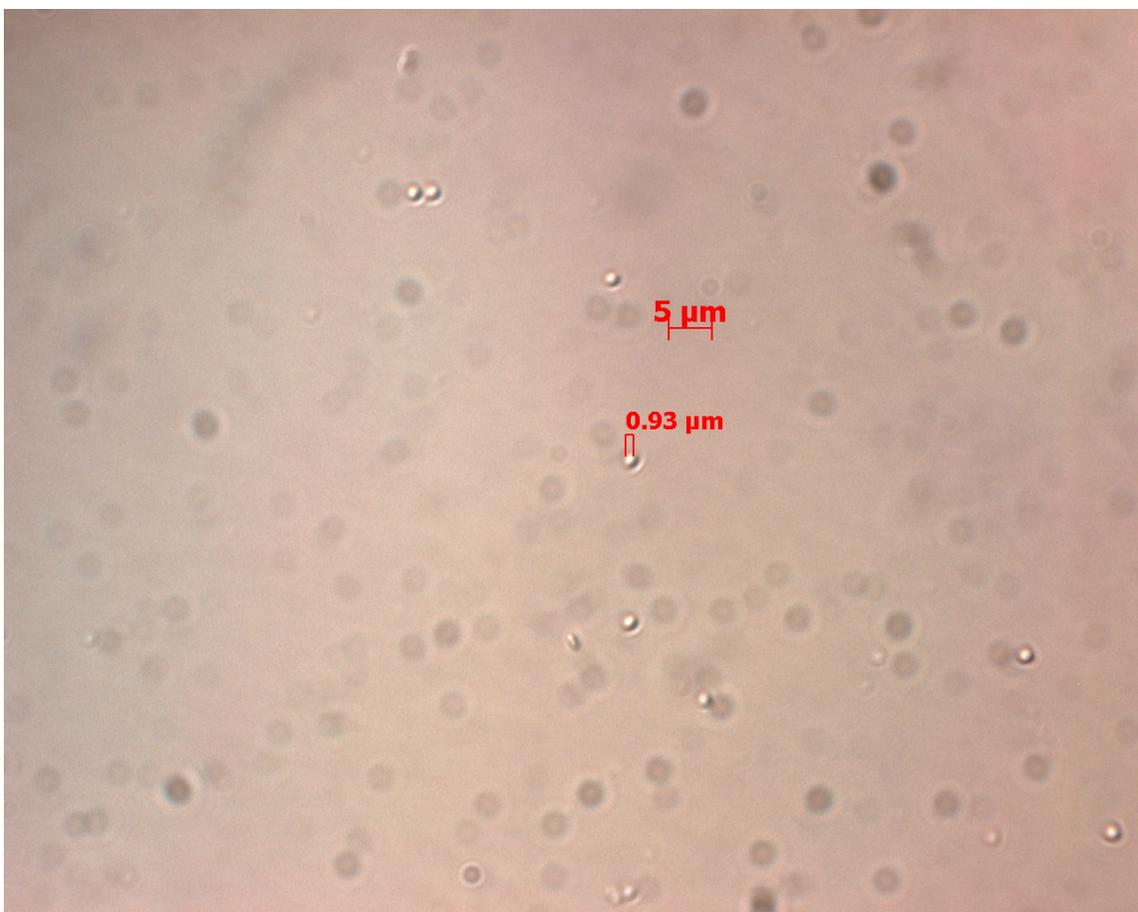


Figure B-1. Mono-dispersed *B. atrophaeus* endospores used for challenge tests.

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 log₁₀ per 100 mL. The test data is presented in Tables B-1 and B-2.

Module Number	Sample Point	Feed		Filtrate	
		Geometric Mean (Oocysts/L)	Log ₁₀	Geometric Mean (Oocysts/L)	Log ₁₀
Module 1	3 Minutes	6.0x10 ⁵	5.78	<1	0.0
	15 Minutes	7.5x10 ⁵	5.88	<1	0.0
	30 Minutes	5.7x10 ⁵	5.76	<1	0.0
	Overall Mean	6.4x10 ⁵	5.81	<1	0.0
Module 2	3 Minutes	3.2x10 ⁵	5.51	<1	0.0
	15 Minutes	5.8x10 ⁵	5.76	<1	0.0
	30 Minutes	5.8x10 ⁵	5.76	<1	0.0
	Overall Mean	4.8x10 ⁵	5.68	<1	0.0
Module 3	3 Minutes	4.8x10 ⁵	5.68	<1	0.0
	15 Minutes	5.8x10 ⁵	5.76	<1	0.0
	30 Minutes	4.1x10 ⁵	5.61	<1	0.0
	Overall Mean	4.9x10 ⁵	5.68	<1	0.0
Module 4	3 Minutes	4.8x10 ⁵	5.68	<1	0.0
	15 Minutes	4.7x10 ⁵	5.67	<1	0.0
	30 Minutes	4.7x10 ⁵	5.67	<1	0.0
	Overall Mean	4.7x10 ⁵	5.67	<1	0.0
Module 5	3 Minutes	5.2x10 ⁵	5.71	<1	0.0
	15 Minutes	4.7x10 ⁵	5.67	<1	0.0
	30 Minutes	5.1x10 ⁵	5.71	<1	0.0
	Overall Mean	5.0x10 ⁵	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 ₁₀	Geometric Mean (CFU/100 mL)	Log ₁₀
Module 1	3 Minutes	1.0x10 ⁷	7.00	2	0.3
	15 Minutes	9.3x10 ⁶	6.97	1	0.0
	30 Minutes	9.0x10 ⁶	6.95	4	0.6
	Overall Mean	9.4x10 ⁶	6.97	2	0.3
Module 2	3 Minutes	6.0x10 ⁶	6.78	<1	0.0
	15 Minutes	7.5x10 ⁶	6.88	3	0.5
	30 Minutes	8.1x10 ⁶	6.91	<1	0.0
	Overall Mean	7.1x10 ⁶	6.85	1	0.2
Module 3	3 Minutes	9.9x10 ⁶	7.00	<1	0.0
	15 Minutes	1.0x10 ⁷	7.00	<1	0.0
	30 Minutes	9.6x10 ⁶	6.98	<1	0.0
	Overall Mean	9.8x10 ⁶	6.99	<1	0.0
Module 4	3 Minutes	1.08x10 ⁷	7.03	<1	0.0
	15 Minutes	6.2x10 ⁶	6.79	6	0.8
	30 Minutes	9.7x10 ⁶	6.99	2	0.3
	Overall Mean	8.7x10 ⁶	6.94	2	0.4
Module 5	3 Minutes	6.3x10 ⁶	6.80	<1	0.0
	15 Minutes	7.4x10 ⁶	6.87	<1	0.0
	30 Minutes	8.0x10 ⁶	6.90	<1	0.0
	Overall Mean	7.2x10 ⁶	6.86	<1	0.0

References

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Appendix C
Challenge Organism Triplicate Counts

Table C-1. <i>B. atrophaeus</i> Triplicate Count Data							
Module	Sample	Feed (CFU/mL)			Filtrate (CFU/100mL)		
		Count 1	Count 2	Count 3	Count 1	Count 2	Count 3
Module 1	Flush	—	—	—	<1	<1	<1
	3 Minutes	6.1E+07	4.1E+07	5.3E+07	<1	<1	<1
	15 minutes	5.7E+07	5.6E+07	6.0E+07	<1	<1	<1
	30 minutes	4.7E+07	4.2E+07	4.9E+07	<1	<1	<1
Module 2	Flush	—	—	—	<1	<1	<1
	3 Minutes	3.8E+07	3.2E+07	4.0E+07	6	2	2
	15 minutes	3.9E+07	4.2E+07	4.1E+07	<1	<1	<1
	30 minutes	5.2E+07	6.4E+07	6.9E+07	2	2	1
Module 3	Flush	—	—	—	<1	<1	<1
	3 Minutes	4.0E+07	4.5E+07	4.3E+07	2	1	<1
	15 minutes	3.3E+07	5.1E+07	3.2E+07	16	<1	<1
	30 minutes	4.0E+07	4.7E+07	4.0E+07	8	13	11
Module 4	Flush	—	—	—	1	<1	<1
	3 Minutes	4.2E+07	5.9E+07	6.0E+07	<1	<1	<1
	15 minutes	3.9E+07	3.0E+07	4.0E+07	1	<1	4
	30 minutes	3.2E+07	4.5E+07	5.0E+07	1	1	1
Module 5	Flush	—	—	—	1	<1	<1
	3 Minutes	6.3E+07	4.9E+07	5.3E+07	4	3	<1
	15 minutes	6.2E+07	4.8E+07	6.1E+07	5	11	12
	30 minutes	4.7E+07	6.5E+07	7.0E+07	3	1	4

Table C-2. MS-2 Triplicate Count Data							
Module	Sample	Feed (PFU/mL)			Filtrate (PFU/mL)		
		Count 1	Count 2	Count 3	Count 1	Count 2	Count 3
Module 1	Flush	—	—	—	<1	<1	<1
	3 Minutes	8.0E+06	7.1E+06	8.6E+06	5600	5600	6700
	15 minutes	4.1E+06	4.3E+07	4.1E+06	5500	3800	4300
	30 minutes	3.4E+06	3.1E+06	4.2E+06	9600	9100	9300
Module 2	Flush	—	—	—	96	151	121
	3 Minutes	4.8E+06	4.2E+06	4.9E+06	1990	1470	2140
	15 minutes	2.7E+06	3.4E+06	3.8E+06	2700	2500	2800
	30 minutes	3.1E+06	3.2E+06	4.3E+06	3000	2500	2800
Module 3	Flush	—	—	—	372	264	324
	3 Minutes	1.72E+06	1.39E+06	1.89E+06	590	820	650
	15 minutes	1.75E+06	1.81E+06	1.48E+06	1160	1230	1410
	30 minutes	3.1E+06	3.4E+06	2.9E+06	1920	1810	1890
Module 4	Flush	—	—	—	372	312	324
	3 Minutes	4.5E+06	4.6E+06	4.0E+06	1320	1490	1370
	15 minutes	3.6E+06	4.4E+06	2.7E+06	2700	3100	3000
	30 minutes	5.7E+06	2.7E+06	3.0E+06	5000	3600	4200
Module 5	Flush	—	—	—	29	14	4
	3 Minutes	1.23E+06	1.41E+06	1.65E+06	400	330	310
	15 minutes	1.13E+06	1.52E+06	1.97E+06	320	290	320
	30 minutes	1.49E+06	1.56E+06	1.72E+06	410	500	290