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Environmental Technology Verification Test/QA plan Drinking Water Systems Center

TEST/QA PLAN FOR THE MICROBIAL SEEDING CHALLENGE STUDY OF THE DOW CHEMICAL COMPANY SFP-2880 ULTRAFILTRATION MODULES FOLLOWING THE REQUIREMENTS OF THE EPA MEMBRANE FILTRATION GUIDANCE MANUAL FOR LT2ESWTR APPROVAL

Prepared by

NSF International

Under a Cooperative Agreement with

U.S. Environmental Protection Agency

ETV ETV ETV

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Abbreviations and Acronyms

ASTM	American Society of Testing Materials
ATCC	American Type Culture Collection
EC	Degrees Celsius
CFU	Colony Forming Units
cm	Centimeter
DWS	Drinking Water Systems
EPA	U. S. Environmental Protection Agency
ETV	Environmental Technology Verification
°F	Degrees Fahrenheit
HPC	Heterotrophic Plate Count
L	Liter
LIMS	Laboratory Information Management System
mg	Milligram
mL	Milliliter
NaOH	Sodium Hydroxide
ND	Non-Detect
NIST	National Institute of Standards and Technology
nm	Nanometer
NSF	NSF International (formerly known as National Sanitation Foundation)
NTU	Nephelometric Turbidity Unit
PFU	Plaque Forming Units
psig	Pounds per Square Inch, Gauge
PSTP	Product-Specific Test Plan
QA	Quality Assurance
QC	Quality Control
QA/QC	Quality Assurance/Quality Control
QAPP	Quality Assurance Project Plan
QMP	Quality Management Plan
RPD	Relative Percent Deviation
SOP	Standard Operating Procedure
TDS	Total Dissolved Solids
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth

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EXECUTIVE SUMMARY

This document is a Test / Quality Assurance Plan (TQAP) for the EPA/NSF Environmental Technology Verification (ETV) Drinking Water Systems (DWS) Center. The purpose of this document is to describe the TQAP for the verification of the Dow Chemical Company SFP-2880 ultrafiltration membrane module for removal of microbial contaminants per the requirements of the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR), as described in the EPA Membrane Filtration Guidance Manual (MFGM). This TQAP describes the performance evaluation test procedure and quality assurance/quality control procedures.

The Dow SFP-2880 ultrafiltration module will be challenged with the MS-2 coliphage virus and endospores of *Bacillus atrophaeus* (ATCC 9372, deposited as *Bacillus subtilis* var. *niger*). The endospores will function as a surrogate for *Cryptosporidium parvum* oocysts. One membrane cartridge will also be challenged with live *C. parvum* oocysts to establish the surrogate relationship between *C. parvum* and *B. atrophaeus* endospores.

The challenge protocol was adapted from the MFGM and the microbial seeding studies in the *ETV Protocol for Equipment Verification Testing for Physical Removal of Microbiological and Particulate Contaminants*. This test plan only applies to microbial challenges. This verification will not address long-term system performance over the life of the membrane, nor will it evaluate cleaning of the membranes, nor any other maintenance and operation.

The experimental design conforms to the sample collection and test procedures for product-specific testing as described in the MFGM. As the ETV Protocol is cited in the EPA's MFGM as an acceptable approach for product-specific testing, the two documents are harmonized in their respective requirements.

NSF International will perform all of the testing activities in their testing laboratory in Ann Arbor, MI. Five membrane modules will be tested. The modules were selected by Dow from different production runs. The membranes will be challenged at the flux specified by Dow. Each module will be challenged for 30 minutes, with feed and filtrate samples collected for challenge organism enumeration at start-up, 15 minutes, and 30 minutes. The modules will be operated at the maximum specified flux of 70 gallons per square foot per day (gfd). The feed and filtrate organism concentrations will be reported as $\log_{10}$ numbers, and $\log_{10}$ reductions will be calculated.

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1.0 Equipment Verification Testing Responsibilities

1.1 Verification Test Site

All testing will be performed at the NSF International Testing Laboratory in Ann Arbor, MI. This laboratory is used for all of the testing activities for NSF certification of drinking water treatment systems, and pool and spa treatment systems.

1.2 Roles and Responsibilities

1.2.1 NSF International

NSF International (NSF) is an independent, not-for-profit organization founded in 1944 for the purpose of developing standards and providing third-party conformity assessment services addressing the needs of governmental agencies, and manufacturers and consumers of products and systems related to public health, safety, and environmental quality.

NSF entered into an agreement on October 1, 2000 with the U. S. Environmental Protection Agency (EPA) to create a Drinking Water Systems (DWS) Center dedicated to technology verifications. NSF manages an Environmental Technology Verification (ETV) Program within the DWS Center for the purpose of providing independent performance evaluations of drinking water technologies. Evaluations are conducted using protocols developed with stakeholder involvement.

NSF will follow the procedures and adhere to the requirements of this TQAP, and will also comply with the data quality requirements in the NSF Drinking Water Systems Center Quality Management Plan (QMP).

The following are the roles and responsibilities of NSF staff involved with the verification testing:

Mike Blumenstein:

- preparation of TQAP;
- provide logistical support, and schedule and coordinate activities in the testing laboratory;
- co-manage, evaluate, and interpret data generated by the verification testing; and
- preparation of the first draft of verification reports and verification statements, and revise these documents after each round of review.

Sal Aridi:

- direct and oversee the NSF Testing Laboratory staff as they perform the testing activities as described in this document; and
- adhere to the QA requirements of this TQAP, associated NSF Standard Operating Procedures (SOP), and the NSF International Laboratories Quality Assurance Manual.

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Rob Donofrio:

- direct and oversee the Microbiology Laboratory staff as they perform microbiological analysis of samples as required by the TQAP; and
- adhere to the QA requirements of this TQAP, associated NSF SOPs, and the NSF International Laboratories Quality Assurance Manual.

Kurt Kneen:

- direct and oversee the Chemistry Laboratory staff as they perform chemical analyses as required by the TQAP; and
- adhere to the QA requirements of this TQAP, associated NSF SOP's, and the NSF International Laboratories Quality Assurance Manual.

Joe Terrell:

- independent review of the TQAP to insure compliance with the requirements of the NSF Drinking Water Systems Center QMP;
- a technical systems audit of the NSF laboratories involved with testing to confirm that the product evaluation, sample management, and sample analyses follow the TQAP and QMP; and
- reviews drafts of the verification reports as needed.

Bruce Bartley:

- co-preparation of TQAP;
- co-manage, evaluate, and interpret data generated by the verification testing;
- co-preparation of the first draft of verification reports and verification statements, and revision of these documents after each round of review;
- designation of an internal technical/engineering reviewer of the TQAP and draft report; and
- co-preparation of the draft and final verification statements.

1.2.3 United States Environmental Protection Agency

The EPA provides leadership in the nation's environmental science, research, education and assessment efforts. The EPA works closely with other federal agencies, state and local governments, and Native American tribes to develop and enforce regulations under existing environmental laws. The agency is responsible for researching and setting national standards for a variety of environmental programs and delegates to states and tribes responsible for issuing permits, and monitoring and enforcing compliance. Where national standards are not met, the EPA can issue sanctions and take other steps to assist the states and tribes in reaching the desired levels of environmental quality. The Agency also works with industries and all levels of government in a wide variety of voluntary pollution prevention programs and energy conservation efforts.

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The following are specific EPA roles and responsibilities:

- QA oversight of NSF International;
- Technical review and QA oversight of TQAP;
- Direct the performance, at the EPA's discretion, of external technical systems audit(s) during the verification testing;
- Review draft verification reports and statements; and
- Final report approval and clearance for signature by the EPA Laboratory Director.

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The Dow SFP-2880 is a polyvinylidene fluoride (PVDF) hollow fiber ultrafiltration membrane module. The module specifications and operating parameters are listed in Table 2-1. The SFP-2880 is a pressure driven module, with the normal operating flow orientation from the outside to the inside of the fibers.

The SFP-2880 is certified to NSF/ANSI Standard 61.

Table 2-1. SFP-2880 Specifications

Parameter	Specification
Dimensions:	
Module outside diameter	8.9 inches (in) (225 millimeters, mm)
Module length	92.9 in (2360 mm)
Module volume	10.3 gallons (gal) (39 liters, L)
Nominal membrane pore size	0.03 µm
Maximum membrane pore size	0.05 µm
Average active membrane area (outer)	829 square feet (ft^2) (77 square meters, m^2)
Operating Limits:	
Filtrate flux range at 25°C	24-70 gallons per square foot per day (gfd) (40-120 $L/m^2/hr$)
Flow range	13.6-40.9 gallons per minute (gpm) (3.1-9.3 m^3/hr)
Operating temperature range	34-104 Fahrenheit (°F) (1-40 Celcius, °C)
Max. inlet module pressure	44 pounds per square inch (psi) (3.0 bar)
Max. transmembrane pressure (TMP)	30 psi (2.1 bar)
Operating pH range	2 – 11
Max. NaOCl	2,000 mg/L
Max. TSS	100 mg/L
Max. Turbidity	300 NTU

Five modules will be tested. The modules were selected by Dow from five different production runs. The module will not be tested in a pilot unit, but rather will be tested in a test rig constructed by NSF. See Section 3.3 for more information about the test rig.

The module design allows for an optional reject line connection, but this port will be closed off for the challenge tests. The modules will be operated in dead-end mode.

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3.1 Experimental Design

The challenge protocol is adapted from the microbial seeding studies in the *ETV Protocol for Equipment Verification Testing for Physical Removal of Microbiological and Particulate Contaminants*, and from the EPA MFGM. The ETV Protocol is cited in the MFGM as an acceptable approach for product-specific testing. This test plan only applies to microbial challenges. This verification will not evaluate cleaning of the membranes, nor any other maintenance and operation.

3.2 Challenge Organisms

All five modules will be challenged with the MS-2 coliphage virus and endospores of *Bacillus atrophaeus* (ATCC 9372, deposited as *Bacillus subtilis* var. *niger*). *B. atrophaeus* was selected to function as a surrogate for *Cryptosporidium parvum*, due to the high cost and lack of availability of suitable numbers of *C. parvum* for challenge testing. The strain of *B. atrophaeus* to be used yields orange colonies with a distinctive morphology on trypicase 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. In addition, one module will be challenged with live *C. parvum* oocysts in order to experimentally confirm that *B. atrophaeus* is a suitable surrogate for *C. parvum*. See Appendix A for further discussion regarding the use of *Bacillus* endospores as a surrogate for *Cryptosporidium*.

The challenge organism suspensions will be injected into the feed water stream with the following target concentrations in the feed water:

- MS-2 – $5x10^5$ plaque forming units per milliliter(PFU/mL);
- *B. atrophaeus* – $1x10^7$ colony forming units (CFU) per 100mL; and
- *C. parvum* – $5x10^5$ oocysts per liter (L).

The MFGM calls for the maximum challenge concentration to be 6.5 $\log_{10}$ above the organism's detection limit ($3.16x10^6$). The goal for the *B. atrophaeus* challenges is to be able to measure log reductions greater than six. Based on previous testing experience and expected organism recovery levels, it is necessary to set the target at approximately 0.5 $\log_{10}$ above the $3.16x10^6$ CFU/100 mL limit to ensure that greater than $1x10^6$ CFU/100 mL will be measured in the feed samples.

3.3 Test Apparatus

The modules will be tested in a test rig constructed specifically for these tests. The test rig construction will conform to the requirements of the MFGM. See Figure 3-1 for a schematic diagram of the test rig to be constructed for testing.

As stated in Section 2.0, the modules will be operated in dead-end mode.

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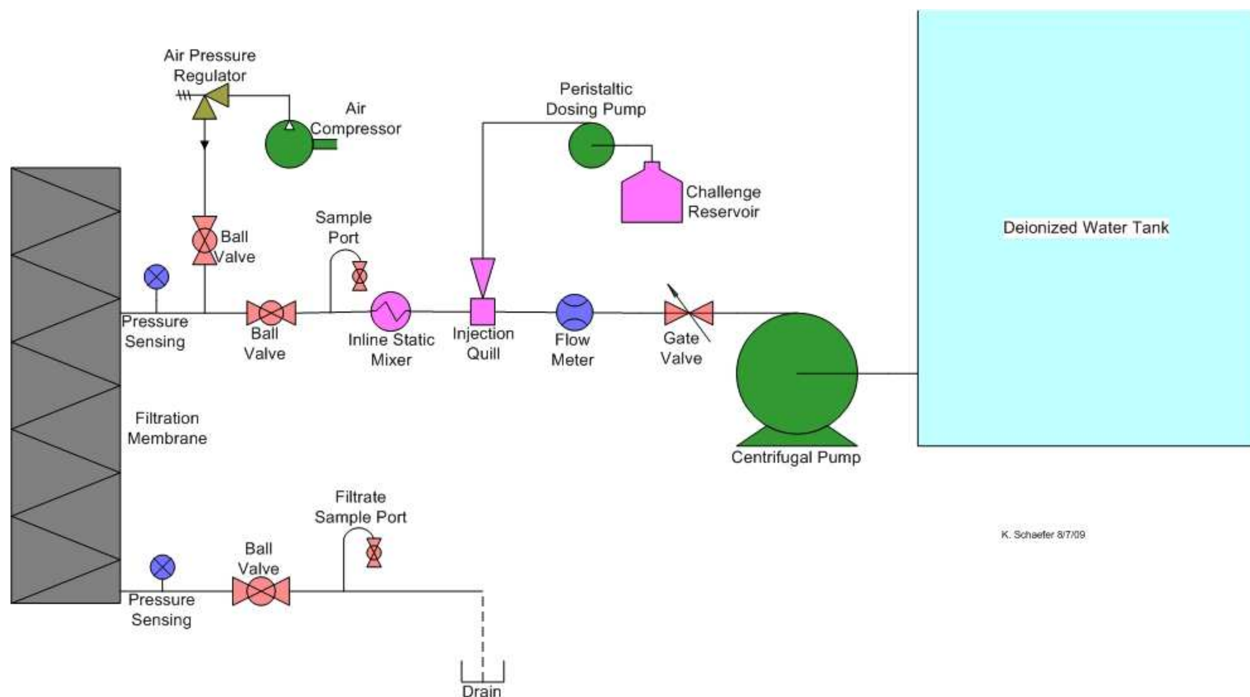

Figure 3-1. Schematic diagram of the test rig to be used for verification testing.

The challenge organisms will be introduced into the feed water by intermittent injection during the challenge tests. Injection and mixing of the organisms will follow the guidelines of the MFGM. Specifically, the stock solution volume for injection will be between 0.5 and 2 percent of the total test solution volume, a chemical metering pump that delivers a steady flow of the challenge solution will be used, and the injection port will include a quill that extends into the middle of the feed pipe.

Feed and filtrate grab samples will be collected from sample ports that also have quills extending into the middle of the pipe, and the sample tap tips will be metal so they can be flame-sterilized prior to sample collection. The feed sample tap will be located at least ten pipe diameters downstream of the injection point, and the test rig will include an in-line static mixer in between the injection and feed sample ports. The feed and filtrate sample ports will be located as close as possible to the membrane modules.

3.4 Test Water Composition

Local tap water treated by carbon filtration, reverse osmosis, ultraviolet disinfection, and deionization will be used as 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.

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Of the above parameters, only total chlorine will be 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.

If necessary, the water will be treated by further mechanical filtration to reduce the concentration of suspended solids to as low as possible.

A water supply tank will filled with the base water, and sodium bicarbonate will be added in sufficient quantity to provide alkalinity at a target of 100 ± 10 mg/L as calcium carbonate. The pH will then be adjusted as necessary with hydrochloric acid or sodium hydroxide to reach the target range of 7.5 ± 0.5.

Feed water samples will be collected prior to each challenge period for analysis of total chlorine, alkalinity, pH, temperature, total dissolved solids, total organic carbon, and turbidity. These samples will be collected prior to injection of the challenge organism.

3.5 Sanitizing the Test Rig

Prior to initiation of testing, and during each module changeout, the test rig will be sanitized using a bleach solution at an appropriate CT. Deionized water shall be used for the sanitization procedure.

3.6 Module Conditioning

Prior to testing, the modules will be conditioned following a procedure supplied by Dow. Immediately prior to testing, each module will also be backflushed per Dow's specifications.

3.7 Membrane Integrity Tests

Before and after each challenge test, each module will undergo a 20-minute pressure decay test to satisfy the non-destructive performance test requirement in Section 3.6 of the MFGM. The test procedure will follow ASTM D6908-03 *Standard Practice for Integrity Testing of Water Filtration Membrane Systems*. The water will be drained from the feed side of the membrane, but not the filtrate side. Air pressure will be applied to the feed side to measure the decay rate. The applied pressure will be measured every minute to chart the pressure decay.

The baseline pressure decay of the test rig will also be measured over 20 minutes and recorded prior to installation of each module. Then, the initial applied pressure for that module's pre-challenge and post-challenge pressure decay tests will be greater than or equal to 20 psig plus the total baseline decay value measured over 20 minutes. This applied pressure will meet the applied pressure resolution requirement of Section 4.2.1 of the MFGM.

3.8 Microbial Challenge Test Procedure

Each of the five SFP-2880 modules submitted for testing will be challenged individually. The test rig will be sanitized with a bleach solution before the start of testing, and as part of the changeout procedure for each module. The target flux for membrane operation will be Dow's

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maximum recommended value of 70 gfd at 25 °C, which equals a flow rate of approximately 40 gpm.

Separate challenge tests will be conducted for each challenge organism, so each module will be tested twice over the course of the testing activities, and one module will be tested a third time with *C. parvum*. The module chosen for the *C. parvum* challenge will be the one with the highest filtrate counts from the *Bacillus* endospores challenge. The testing laboratory expects to run both the MS-2 and *Bacillus* challenges in the same day, so that one module is tested per day.

The modules will be "brand new" when challenged. There will be no seasoning period, other than that specified by Dow to sufficiently rinse out the membrane preservative and wet the membranes.

Each membrane will be individually plumbed to the test rig after the rig has been sanitized and rinsed. If it is the first time the module is installed, it will be flushed per Dow's flushing and conditioning procedure. If the module has already been tested once (or twice in the case of the module for the *C. parvum* test), the module will only be forward flushed at 40 gpm for one minute. Immediately prior to testing, each module will be backflushed for one minute at a flow rate of 40 gpm.

The next step will be the pre-challenge pressure decay test. See Section 3.7 for the pressure decay test procedure. After the pressure decay test is complete, the test water feed (minus challenge organism injection) will be resumed at 40 gpm. After an additional minute of membrane flushing, a negative control filtrate flush sample will be collected for challenge organism enumeration. During this flush, also collect an additional filtrate sample to serve as the matrix spike sample, and adjust the flow rate and feed/filtrate pressures as necessary, to prepare for the challenge test.

Each challenge test will be approximately 35 minutes in length. As discussed in Section 3.3, the challenge organisms will be intermittently injected into the feed stream prior to, and during sample collection. Sections 3.10.2, 3.10.4, and 3.12.4 of the MFGM describe the requirements for the challenge test sampling plan. The MFGM requires 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 allow for establishment of 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." Dow's specification sheet for the SFP-2880 gives the module volume as 10.3 gal. It is assumed that this volume is the total water holding volume of the module, not just the volume of the feed side of the membranes. As such, its use as the hold-up volume will add a safety factor to the hold up volume calculation.

The MFGM also specifies that the challenge organisms are injected at least 10 pipe diameters upstream of the feed sample tap, and that the feed sample tap should be as close as possible to the modules. The inlet and outlet fittings on the SFP-2880 module are 2 in (DN50), so the pipe to be used for the test rig will also be 2 inches in diameter. Therefore, the injection point must be at least 20 in upstream of the feed sample tap. The test rig has not yet been constructed as of this

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writing, so the test plan will speculate here about the expected hold-up volume of the test rig. The injection point will be at most 36 in from the feed sample tap. Thirty-six inches of 2-inch diameter pipe has a volume of 113 in^3, which translates into 0.49 gal. The maximum expected pipe volume plus the module volume gives a hold-up volume of approximately 10.8 gal, which will be rounded up to 11 gal here for simplicity. If the hold-up volume is 11 gal, then the equilibrium volume is 33 gal. The challenge flow rate will be approximately 40 gpm, so the challenge organisms will be injected for 1 minute prior to sampling to meet the requirement of passing the equilibrium volume.

The challenge organism will be injected into the feed stream at start-up, after 15 minutes of operation, and after 30 minutes of operation. After 1 minute of injection, grab samples will be collected from the feed and filtrate sample taps. The sample taps will be flame sterilized prior to sample collection. Also, at least 100 mL will be collected and discarded prior to sample collection to flush the taps. After sample collection is complete, injection will be turned off and clean water will be pumped through the modules until the next sampling point.

Log reduction values (LRV) will be calculated for each set of feed and filtrate samples.

The test procedure can be summarized as follows:

1. Sanitize the test rig with deionized water spiked with an appropriate amount of bleach.
2. Install and condition the module, or flush for one minute.
3. Backflush module at 40 gpm for 60 seconds.
4. Conduct the pre-challenge pressure decay test.
5. Conduct the microbial challenge test
    a. Flush the module for 1 minute, then collect the filtrate flush and matrix spike samples.
    b. Adjust the flow and pressure if needed.
    c. Collect feed samples for the water quality analyses.
    d. Begin injection of the challenge organism suspension.
    e. Inject the challenge organism for at least one minute, then collect the required volumes of feed, then filtrate for microbial analysis. Flame sterilize the sample taps prior to sample collection. Flush the sample taps with at least 100 mL prior to beginning sample collection.
    f. After sample collection is complete, turn off injection.
    g. Operate the module using the feed water minus the challenge injection until the next sampling point.
    h. Repeat steps d through g after 15 minutes and 30 minutes of elapsed module operation time.
6. Conduct the post-challenge pressure decay test.

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4.1 Introduction

This TQAP specifies procedures that will be used to ensure accurate documentation of UF module performance. Careful adherence to these procedures and to the analytical procedures will result in verifiable performance data.

4.2 Analytical Methods

A list of laboratory analytical methods for all parameters but MS-2 enumeration can be found in Table 4.1. The analytical method for MS-2 is explained below the table.

Table 4.1 Analytical Methods for Laboratory Analyses

Parameter	Method	NSF Reporting Limit	Lab Accuracy (% Recovery)	Lab Precision (%RPD[1])	Holding Time	Sample Container	Sample Preservation
Alkalinity (total)	SM 2320B[2]	5 mg/L	90-110	≤ 13	14 days	1 L plastic	none
pH	SM 4500-H^+ B		± 0.1 units	≤ 10	(3)	NA	none
TDS	SM 2540 C	5 mg/L	90-110	≤ 10	7 days	1 L plastic	none
Total Chlorine	SM 4500-Cl G	0.05 mg/L	90-110	≤ 10	(3)	NA	none
Turbidity	SM 2130 B	0.1 NTU[4]	95-105		(3)	NA	none
MS-2	see below	1 PFU/mL	NA	NA	8 hours	125 mL plastic	polysorbate 20 (Tween), store at 3 ± 2 °C
B. atrophaeus Endospores	SM9218[5]	1 CFU/100 mL	NA	NA	30 hours	125 mL plastic for feed, 1 L plastic for filtrate	polysorbate 20 (Tween), store at 3 ± 2 °C
C. parvum oocysts	EPA 1623	1 oocyst/L	NA	NA	72 hours	1 L plastic[6]	polysorbate 20 (Tween), store at 3 ± 2 °C

(1) RPD = Relative Percent Deviation
(2) SM = Standard Methods
(3) Immediate analysis required
(4) NTU = Nephelometric Turbidity Unit
(5) Trypticase soy agar (TSA) will be 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.
(6) For the required triplicate analyses, plus backup samples, the Microbiology Laboratory will need six 1 L bottles of filtrate at each sample point. Two 1 L bottles of the feed will be needed. The feed and filtrate samples should be collected into a single sterile container, and the 1 L bottles filled from these volumes.

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

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- Alkalinity – SmartChem Discrete Analyzer;
- pH – Orion EA 940 pH/ISE meter;
- Temperature – Fluke 51 II digital thermometer;
- Total Chlorine – Hach DR/2800 spectrophotometer using AccuVac vials; and
- Turbidity – Hach 2100P turbidimeter.

4.2.1 Sample processing, and enumeration of MS-2 coliphages:

One milliliter volumes of the feed samples will be serially diluted for enumeration. One milliliter volumes of the filtrate samples will be both enumerated directly and serially diluted for enumeration. The one mL volumes are added to tubes containing the host *E. coli* in tryptic soy broth (TSB). The tube is vortexed for 30 seconds, and then 4 mL of molten, tempered 1% tryptic soy agar (TSA) is added to the tube. This mixture is then poured over a TSA plate, and the plate is incubated at 35 °C for 18-24 hours. The viral plaques will be counted using a Quebec Colony Counter.

4.3 Analytical QA/QC Procedures

Accuracy and precision of sample analyses shall be ensured through the following measures:

- Alkalinity – A certified QC sample is analyzed each day. The acceptable recovery limit is that specified with the sample.
- pH – Three-point calibration (4, 7, 10) of the pH meter used to give the reportable data shall be conducted daily using traceable buffers. The accuracy of the calibration shall be checked daily with a pH 8.00 buffer. The pH reading for the buffer shall be within 10% of its true value. The precision of the meter shall be checked daily using duplicate synthetic drinking water samples. The RPD of the duplicate samples shall be less than 10%.
- TDS – A QC sample is analyzed with each sample batch. The percent recovery must be within 10%, or the QC sample manufacturer's specified limits. Also, one blank (empty evaporating dish) is run with each batch, and must be within 0.5 mg of original weight. Ten percent of samples are analyzed in duplicate, and should agree with 5% of average weight (10% RPD).
- Temperature – The thermometer used to give the reportable data shall have a scale marked for every 0.1ºC. The thermometer is calibrated yearly using a Hart Scientific Dry Well Calibrator Model 9105.
- Total chlorine – The calibration of the chlorine meter shall be checked daily using a DI water sample (blank), and three QC standards. The measured QC standard values shall be within 10% of their true values. The precision of the meter shall be checked daily by duplicate analysis of synthetic drinking water samples. The RPD of the duplicate samples shall be less than 10%.
- Turbidity – The turbidimeter shall be calibrated as needed according to the manufacturer's instructions with formazin standards. Accuracy shall be checked daily

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with a secondary Gelex standard. The calibration check shall give readings within 5% of the true value. The precision of the meter shall be checked daily by duplicate analysis of synthetic drinking water samples. The RPD of the duplicate samples shall be less than 10%.

- Sample processing and enumeration of MS-2
  - Samples will be stored in the dark at 3 ± 2 °C until analyzed.
  - All samples will be analyzed in triplicate.
  - All batches of media will be checked for sterility and for positive growth response.
  - Membrane filters and dilution water will also be checked for sterility.
  - Cultures will be checked for purity.
- Sample processing and enumeration of *B. subtilis* endospores.
  - Samples will be stored in the dark at 3 ± 2 °C until analyzed.
  - All samples will be analyzed in triplicate.
  - All batches of media will be checked for sterility and for positive growth response.
  - Membrane filters and dilution water will also be checked for sterility.
- Sample processing and enumeration of *C. parvum*
  - Samples will be stored in the dark at 3 ± 2 °C until analyzed.
  - All samples will be analyzed in triplicate.
  - A matrix spike (MS) sample will be processed and enumerated with every set of samples. The percent recovery of the oocysts will be measured using the equation in Section 5.3.2. The upper and lower control limits for percent recovery are defined as the mean percent recovery from the last 20 recovery analyses ± 3 standard deviations. The NSF Microbiology Laboratory's current percent recovery control limits are 53% to 135%. The mean recovery is 94.3%. New control limits are calculated after every 10 recovery analyses. The matrix spike is also used as the positive antigen control for the Crypto-a-glo™ antibody.

Please note that NSF analyzes many samples for these parameters every day. The samples for alkalinity and TDS will be included in larger sample batches. Duplicate sample analysis requirements apply to the whole batch, so NSF may not perform duplicate analysis on 10% of samples from this test.

4.4 Sample Handling

All samples not immediately analyzed will be labeled with unique identification numbers. These identification numbers will be entered into the NSF Laboratory Information Management System (LIMS), and will appear on the NSF lab reports for the tests. All challenge organism samples will be stored in the dark at 3 ± 2 °C until processed for analysis.

Chlorine, pH, turbidity will be measured immediately after sample collection.

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4.5 Documentation

All laboratory activities will be thoroughly documented using lab bench sheets and NSF LIMS laboratory reports.

NSF will be responsible for maintaining all documentation. Lab bench sheets will be used to record all water treatment equipment operating data. Each page will be labeled with the project name and number. Errors will have one line drawn through them and this line will be initialed and dated.

Any deviations from the approved final TQAP will be thoroughly documented at the time of inspection and in the verification report.

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5.1 Introduction

The Quality Assurance Project Plan (QAPP) for this verification test specifies procedures that will be used to ensure data quality and integrity. Careful adherence to these procedures will ensure that data generated from the verification testing will provide sound analytical results that can serve as the basis for the performance verification.

This section outlines steps that will be taken by NSF to ensure that data resulting from verification testing is of known quality and that a sufficient number of critical measurements are taken.

5.2 Quality Assurance Responsibilities

A number of individuals will be responsible for test equipment operation, sampling, and analysis QA/QC throughout the verification testing. Primary responsibility for ensuring that these activities comply with the QA/QC requirements of this TQAP rests with the supervisors of the individual NSF laboratories.

NSF QA/QC staff will review the raw data records for compliance with QA/QC requirements. NSF ETV staff will check 100% of the raw data records against the reported results in the LIMS reports.

5.3 Data Quality Indicators

The data obtained during the verification testing must be of sound quality for conclusions to be drawn on the treatment equipment. For all verification activities, data quality parameters must be established based on the proposed end uses of the data. These parameters include five indicators of data quality: representativeness, accuracy, precision, statistical uncertainty, and completeness.

5.3.1 Representativeness

Representativeness refers to the degree to which the data accurately and precisely represent the conditions or characteristics of the parameter represented by the data, or the expected performance of the RO system under normal use conditions. Representativeness will be ensured by executing consistent sample collection protocols, including timing of sample collection, sampling procedures, and sample preservation. Representativeness will also be ensured by using each analytical method at its optimum capability to provide the most accurate and precise measurements possible.

5.3.2. Accuracy

Accuracy is a measure of the deviation of the analytical value from the true value. Since true values for samples can never be known, accuracy measurements are made through analysis of certified standards or QC samples of a known quantity.

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Accuracy will be maintained through the following items:

- Maintaining consistent sample collection procedures, including sample locations, timing of sample collection, and sampling procedures;
- Calibrated instruments; and
- Laboratory control samples (e.g., method blanks, duplicates, matrix spikes, matrix spike duplicates, and performance evaluation samples).

Recoveries for spiked samples will be calculated in the following manner:

$$\text{Percent Recovery} = \frac{100*(SSR - SR)}{SA}$$

where: SSR = spiked sample result
SR = sample result
SA = spike amount added

Recoveries for laboratory control samples are calculated as follows:

$$\text{Percent Recovery} = \frac{100*(Found\ Concentration)}{True\ Concentration}$$

For acceptable analytical accuracy, the recoveries must be within control limits. The NSF laboratory's minimum acceptable accuracy for each parameter is listed in Table 4.1.
The accuracy of the benchtop chlorine, pH, and turbidity meters will be checked daily during the calibration procedures using certified check standards. For samples analyzed in batches (alkalinity and TDS), certified QC samples will be run with each batch.

5.3.3 Precision

Precision refers to the degree of mutual agreement among individual measurements and provides an estimate of random error. Precision will be measured through duplicate sample analysis. One sample per batch will be analyzed in duplicate for the TDS and alkalinity analyses. To check the precision of the benchtop chlorine, pH, and turbidity meters, duplicate synthetic drinking water samples will be analyzed daily. Precision of the duplicate analyses will be measured by use of the following equation to calculate RPD:

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

where:

S_1 = sample analysis result; and
S_2 = sample duplicate analysis result.

Acceptable RPD values for each parameter are given in Table 4.1.

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5.3.4 Statistical Uncertainty

Statistical uncertainty of the triplicate challenge organism counts will be evaluated using Microsoft® Excel 2003 to calculate the 95% confidence intervals. The following formula will be employed for confidence interval calculation:

$$\text{confidence interval} = \overline{X} \pm t_{1-\frac{\alpha}{2}} \left(S/\sqrt{n}\right)$$

where: $\overline{X}$ is the sample mean;
S is the sample standard deviation;
n is the number independent measures included in the data set;
t is the Student's t distribution value with n-1 degrees of freedom; and
∀ is the significance level, defined for 95% confidence as: 1 - 0.95 = 0.05.

5.3.5 Completeness

Completeness refers to the amount of data collected from a measurement process compared to the amount that was expected to be obtained. Completeness refers to the proportion of valid, acceptable data generated using each method. This portion of the required data for the selected test plan will be reported at the conclusion of each testing period.

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. The following chart illustrates the completeness objectives for performance parameter and/or method based on the sample frequency:

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;
T = total number of measurements.

Retesting may be required if the completeness objectives are not met.

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The following are examples of instances that might cause a sample analyses to be incomplete:

- Instrument failure;
- Calibration requirement not being met; or
- Elevated analyte levels in the method blank.

5.4 Data Validation and Reporting

To maintain good data quality, specific procedures will be followed during data validation, and reporting. These procedures are detailed below.

5.4.1 Data Validation

For the analytical data:

- NSF ETV staff will review calculations and inspect laboratory logbooks and data sheets to verify accuracy of data recording and sampling;
- The NSF QA/QC department will verify that all instrument systems are in control and that QA objectives for accuracy, precision, and method detection limits have been met; and
- NSF QA and ETV staff will review the raw data records for compliance with QC requirements and check 100% of the data against the reported results from the LIMS reports.

Should QC data be outside of control limits, the analytical laboratory supervisor will investigate the cause of the problem, and discussion of the problem will be included in the final report. Depending on the severity of the problem, the data in question may be flagged, or not reported.

5.4.2 Data Reporting

The data to be reported will be the feed and treated water microorganism counts, log reductions, and the water chemistry data. The QC data, such as calibrations, blanks and reference samples will be not be reported, but will be kept on file at NSF.

5.5 Testing Inspections

NSF QA staff will conduct an audit of the laboratory during testing to ensure compliance with the test procedures and requirements of this TQAP. The results of all audits will be reported to the NSF ETV staff. Throughout testing, ETV staff will carry out random spot inspections. Any variances will be reported to NSF QA staff.

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6.1 Data Management and Analysis

All operational and analytical data will be gathered and included in the Final ETV Report. The data will consist of results of analyses and measurements and QA/QC reports.

The data management system for this verification involves the use of the NSF LIMS computer system, spreadsheet software and manual recording of system operating parameters.

6.2 Work Plan

The following is the work plan for data management:

- Laboratory personnel will record equipment operation, water quality and analytical data by hand on bench sheets.
- All bench sheet entries will be made in water-insoluble ink.
- All corrections on the bench sheets will be made by placing one line through the erroneous information. Any corrections will be dated and initialed by the lab personnel making the correction.
- Pertinent information from the bench sheets will be entered into the LIMS system. When the test is complete, a preliminary report will be generated. The preliminary report will be reviewed by the manager of any laboratory that entered data. Once the preliminary report is approved, a final laboratory report will be generated and given to ETV staff.

The database for this verification testing program will be set up in the form of custom-designed spreadsheets. Pertinent data from the LIMS reports will be entered into the appropriate spreadsheets. All recorded calculations will also be checked at this time. Following data entry, the spreadsheet will be printed out and the printout checked against the LIMS report.

6.3 Performance Reporting

Microorganism removal by the UF module will be evaluated through log reduction calculations. All challenge organism samples will be analyzed in triplicate, so the geometric mean of each triplicate set of results will be used for the calculations.

6.4 Report of Equipment Testing

The report will be issued in draft form for review prior to final publication. The reports will be prepared by NSF and will consist of the following:

- Introduction;
- Description and Identification of Product Tested;
- Procedures and Methods Used in Testing;
- Results and Discussion, including QA/QC discussion; and
- References;

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This report will be prepared using Microsoft Word® 2003.

NSF ETV staff will prepare the first draft of the Verification Report and Verification Statement. These documents will be reviewed by the NSF QA officer, and then will be sent to an outside technical advisor for review. NSF will also send the draft documents to the EPA for review concurrent with the technical advisor review.

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Appendix A
***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 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 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 µm cm V^{-1} s^{-1} for *C. parvum*, and -1.9 to -2.2 µm cm $V^{-1}s^{-1}$ 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 $1x10^9$ 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.

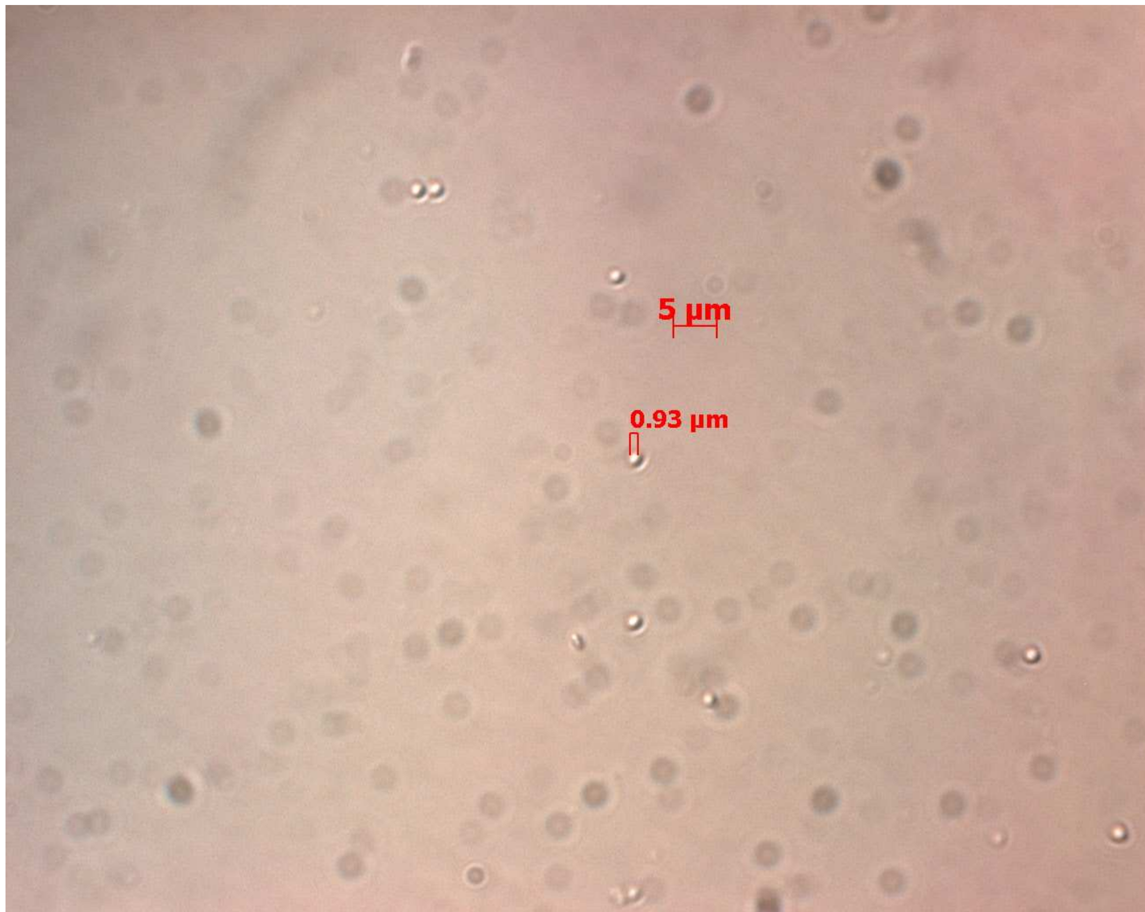

Figure B-1. Mono-dispersed *B. atrophaeus* endospores used for challenge tests.

References

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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.

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Appendix B
Dow Flushing Procedure

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