Draft 1.0 (10/12/10)

Environmental Technology Verification Test/QA plan Drinking Water Systems Center

TEST/QA PLAN FOR VALIDATING THE DOW CHEMICAL COMPANY SFD-2880 ULTRAFILTRATION MEMBRANE MODULE FOR VIRUS REDUCTION FOLLOWING THE DEPARTMENT OF HEALTH VICTORIA (AUSTRALIA) DRAFT GUIDELINES FOR VALIDATING TREATMENT PROCESSES FOR PATHOGEN REDUCTION

Prepared by

NSF International

Under a Cooperative Agreement with

U.S. Environmental Protection Agency

Contents

Tables

Figures

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
µS	microSiemens

EXECUTIVE SUMMARY

This document is a Test/Quality Assurance Plan (TQAP) for the U.S. Environmenal Protection Agency (EPA)/NSF Environmental Technology Verification (ETV) Drinking Water Systems (DWS) Center. The purpose of this document is to describe the TQAP for a cut fiber challenge study requested by Dow Chemical Company for their SFD-2880 ultrafiltration membrane module. The challenge tests shall follow the requirements of the Department of Health Victoria (Australia) *Draft guidelines for validating treatment processes for pathogen reduction*. These requirements are largely based on the EPA's Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) and Membrane Filtration Guidance Manual (MFGM). This TQAP describes the performance evaluation test procedure and quality assurance/quality control procedures.

Five Dow SFD-2880 ultrafiltration membrane modules shall be challenged with the MS2 coliphage virus. The five modules shall first be challenged as fully intact membranes, then one fiber in each module shall be cut and a second round of challenges shall be conducted to evaluate the performance of compromised modules. The five modules shall be supplied by Dow from five different product lots. 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.

NSF International shall perform all of the testing activities in their testing laboratory in Ann Arbor, MI. The modules shall be challenged at the maximum design flux specified by Dow. Each module shall be challenged for approximately 30 minutes, with feed and filtrate samples collected for challenge organism enumeration at start-up after achieving steady state operation, then again after 15 minutes and 30 minutes of operation. A concentrated suspension of MS2 shall be injected into the feed stream immediately prior to and during sample collection following the requirements of the MFGM.

1.0 Equipment Verification Testing Responsibilities

1.1 Verification Test Site

All testing shall 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 is accredited by the American National Standards Institute (ANSI) and Standards Council of Canada (SCC) for product certifications according to the requirements of ISO Guide 65 – General Requirements for Bodies Operating Product Certification Systems. NSF's testing laboratory is accredited by SCC and the International Accreditation Service to ISO 17025.

NSF also currently has a pending approval with JAS/ANZ for the Australian WaterMark, and JAS/ANZ also has a Multilateral Recognition Arrangement with ANSI for Product Certification Bodies, through the Pacific Accreditation Cooperation agreement.

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 shall follow the procedures and adhere to the requirements of this TQAP, and shall 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.

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.

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.

2.0 Equipment Description

The Dow SFD-2880 is a polyvinylidene fluoride (PVDF) hollow fiber ultrafiltration membrane module. The module specifications and operating parameters are listed in Table 2-1. A diagram of the module is displayed in Figure 2-1. The SFD-2880 is a pressure driven module, with the normal operating flow orientation from the outside to the inside of the fibers. The SFD-2880 is certified to NSF/ANSI Standard 61.

Table 2-1. SFD-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

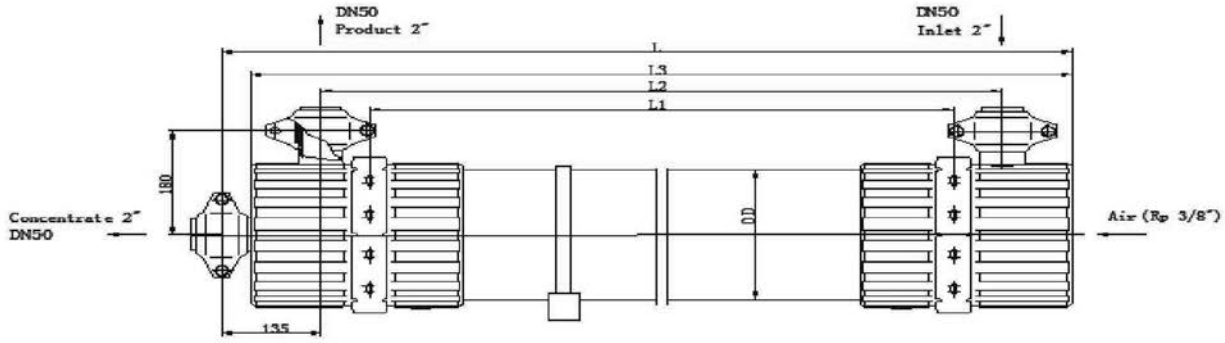

Figure 2-1. Diagram of the SFD-2880 UF module.

Five modules shall be tested. The modules were selected by Dow from five different production runs. The modules will not be tested in a pilot unit, but rather shall 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 shall be closed off for the challenge tests. The modules shall be operated in dead-end mode as this is a worst-case operation scenario.

3.0 Experimental Design

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.

Five intact modules shall be challenged with MS2 coliphage virus, then one membrane fiber in each module shall be cut, and the challenge tests conducted again. NSF International shall perform all of the testing activities in their testing laboratory in Ann Arbor, MI. The modules shall be challenged at the maximum design flux specified by Dow. Each module shall be challenged for approximately 30 minutes, with feed and filtrate samples collected for challenge organism enumeration at start-up after achieving steady state operation, then again after 15 minutes and 30 minutes of operation. A concentrated suspension of MS2 shall be injected into the feed stream immediately prior to and during sample collection following the requirements of the MFGM.

3.2 Challenge Organism

The modules shall be challenged with the MS2 coliphage virus as a surrogate for enteric viruses. MS2 is generally accepted as an enteric virus surrogate for size-exclusion technologies due to its small size (approximately 22-26 nanometers). The target feed concentration shall be $5x10^5$ plaque forming units per milliliter (PFU/mL).

3.3 Test Apparatus

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

MS2 shall be introduced into the feed water by intermittent injection during the challenge tests. Injection and mixing of the organisms shall follow the guidelines of the MFGM. Specifically, the stock solution volume for injection shall 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 shall be used, and the injection port shall include a quill that extends into the middle of the feed pipe.

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

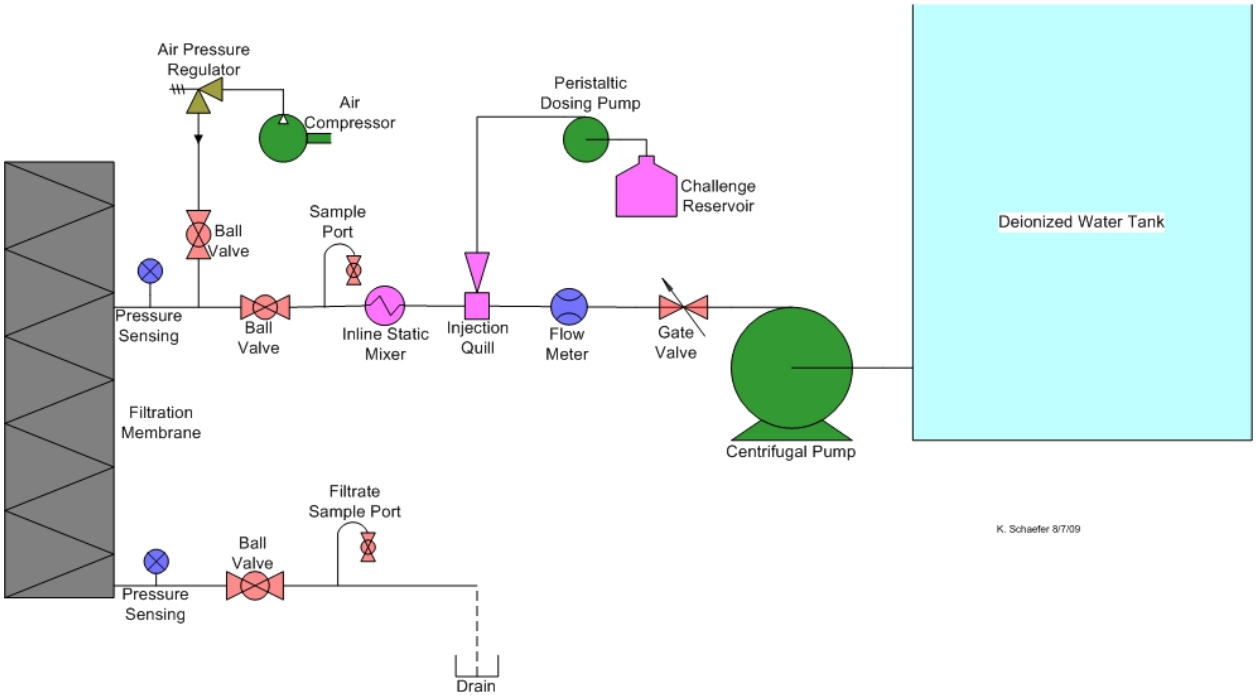

Figure 3-1. Schematic diagram of the test rig to be used for verification testing.

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

If necessary, the water shall be treated by further mechanical filtration to reduce the concentration of suspended solids to as low as possible.
A water supply tank will be filled with the base water, and sodium bicarbonate shall 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 shall 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 shall be collected prior to injection of MS2.

3.5 Sanitizing the Test Rig

Prior to initiation of testing, and during each module changeout, the test rig shall 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 shall be conditioned following a procedure supplied by Dow. Immediately prior to testing, each module shall also be backflushed per Dow's specifications.

3.7 Membrane Integrity Tests

Prior to initiation of testing activities, a Dow representative will visit NSF to inspect the modules for broken fibers, or other integrity breaches. Any fibers deemed to be compromised shall be plugged. After inspection and repair of each module, a pressure decay test shall be conducted. If Dow is satisfied with the pressure decay rate, the module shall be officially handed over to NSF for testing.

Immediately before and after each challenge test, each module shall undergo a 20-minute pressure decay test to demonstrate continued membrane integrity. The pressure decay tests will also be conducted correlate a pressure decay rate to the cut fiber scenario. This test will also serve to check for any other membrane fiber integrity problems that may need to be repaired to ensure that the modules only have one cut fiber and no other holes or fiber breaks. The test procedure shall follow ASTM D6908-03 *Standard Practice for Integrity Testing of Water Filtration Membrane Systems*. The water shall be drained from the feed side of the membrane, but not the filtrate side. Approximately 20 psig of air pressure shall be applied to the feed side to measure the decay rate. This pressure meets the 3µm resolution requirement for the non-destructive performance test, as described in Section 4.2 of the MFGM. The applied pressure shall be measured every minute to chart the pressure decay. The filtrate side end cap shall be removed for the test, so that bubbles could be observed to identify any additional fibers with leaks. Removal of the end cap is permissible for a module of the design employed by Dow for the SFD-2880 because it does not provide any critical seals between the feed and filtrate, or filtrate and reject.

The baseline pressure decay of the pressurized test rig plumbing shall also be measured over 20 minutes immediately prior to the pre-challenge pressure decay test.

3.8 Microbial Challenge Test Procedure

Each of the modules shall be challenged individually. The test rig shall 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 shall be Dow's maximum recommended value of 70 gfd at 25 °C, which equals a flow rate of approximately 40 gpm.

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

Each membrane shall be individually plumbed to the test rig after the rig has been sanitized and rinsed. Immediately prior to testing, each module shall be backflushed for one minute at a flow rate of 40 gpm.

The next step shall 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) shall be resumed at 40 gpm. After at least one minute of membrane forward flushing, two feed and two filtrate samples shall be collected. One sample of each process stream shall serve as negative controls, and be enumerated for MS2. The second sample pair shall be spiked with MS2 to serve as positive controls. The testing engineer shall spike these samples with a measured aliquot of the challenge suspension immediately after collection, and the spiked samples shall be submitted to the NSF Microbiology Laboratory with the other samples from that challenge test. This will verify that the MS2 is stable in the feed and filtrate waters over the course of the test, and up to the time that the samples are processed by the Microbiology Laboratory.

Each challenge test shall be approximately 35 minutes in length. MS2 shall be injected into the feed stream at start-up, after 15 minutes of operation, and after 30 minutes of operation. The MS2 suspension shall be constantly stirred using a magnetic stir bar. 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 spiked with MS2 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 SFD-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 holdup 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 SFD-2880 module are 2 in (DN50), so the pipe to be used for the test rig shall 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 writing, so the test plan will speculate here about the expected hold-up volume of the test rig. The injection point shall 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 shall 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 shall be approximately 40 gpm, so the MS2 suspension shall be injected for at least one minute prior to sampling to meet the requirement of passing the equilibrium volume.

After one minute of injection, grab samples shall be collected from the feed and filtrate sample taps. The sample taps shall be flame sterilized prior to sample collection. Also, at least 100 mL shall be collected and discarded prior to sample collection to flush the taps. After sample collection is complete, MS2 injection shall be stopped, and clean water shall be pumped through the modules until the next sampling point.

At the conclusion of each challenge test, a post-challenge pressure decay test shall be conducted per Section 3.7.

Log reduction values (LRV) shall 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 if conditioning is already complete, forward flush for one minute.
3. Backflush module at 40 gpm for one minute.
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.

3.9 UF Fiber Cutting Procedure

To cut a fiber in each module, Dow will instruct NSF to remove the feed end cap to access the fibers in the center feed channel. A fiber in each module will be cut with a small pair of scissors as close as possible to the fiber – potting resin interface to mimic a worst-case fiber break scenario.

4.0 Laboratory Operations Procedures

4.1 Introduction

This TQAP specifies procedures that shall 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 can be found in Table 4.1. The analytical method for MS2 is explained in Appendix A.

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
MS2	Appendix A	1 PFU/mL	NA	NA	8 hours	125 mL plastic	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

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

- 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.

4.3 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 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 MS2
    - Samples shall be stored in the dark at 3 ± 2 °C until analyzed;
    - All samples shall be analyzed in triplicate;
    - Feed water samples shall be spiked with MS2 to measure recovery of the organism;
    - All batches of media shall be checked for sterility and for positive growth response;
    - Membrane filters and dilution water shall also be checked for sterility; and
    - Cultures shall be checked for purity.

Please note that NSF analyzes many samples for these parameters every day. The samples for alkalinity and TDS shall 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

Samples collected for measurement of pH, temperature, total chlorine, and turbidity shall be measured in the testing laboratory immediately after collection, and so will not be labeled. The samples collected for alkalinity, TDS, and MS2 analysis will be delivered to other groups within the NSF Laboratory, so they shall be labeled with unique identification numbers. These identification numbers shall 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 shall be stored in the dark at 3 ± 2 °C until processed for analysis.

4.5 Documentation

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

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

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

5.0 Quality Assurance Project Plan

5.1 Introduction

The Quality Assurance Project Plan (QAPP) for this verification test specifies procedures that shall be used to ensure data quality and integrity. Careful adherence to these procedures shall 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 shall 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 shall 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 shall review the raw data records for compliance with QA/QC requirements. NSF ETV staff shall 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 shall be ensured by executing consistent sample collection protocols, including timing of sample collection, sampling procedures, and sample preservation. Representativeness shall 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.

Accuracy shall 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 shall 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:

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

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 shall be checked daily during the calibration procedures using certified check standards. For samples analyzed in batches (alkalinity and TDS), certified QC samples shall 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 shall be measured through duplicate sample analysis. One sample per batch shall 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 shall be analyzed daily. Precision of the duplicate analyses shall 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.

5.3.4 Statistical Uncertainty

Statistical uncertainty of the triplicate challenge organism counts shall be evaluated using Microsoft® Excel 2003 to calculate the 95% confidence intervals. The following formula shall 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
$\forall$ 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 shall 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; and
T = total number of measurements.

Retesting may be required if the completeness objectives are not met.
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 shall be followed during data validation, and reporting. These procedures are detailed below.

5.4.1 Data Validation

For the analytical data:

- NSF ETV staff shall review calculations and inspect laboratory logbooks and data sheets to verify accuracy of data recording and sampling;
- The NSF QA/QC department shall 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 shall 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 shall investigate the cause of the problem, and discussion of the problem shall 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 shall 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 shall be not be reported, but shall be kept on file at NSF.

5.5 Testing Inspections

NSF QA staff shall 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 shall be reported to the NSF ETV staff. Throughout testing, ETV staff shall carry out random spot inspections. Any variances shall be reported to NSF QA staff.

6.0 Data Management, Analysis, and Reporting

6.1 Data Management and Analysis

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

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

6.3 Performance Reporting

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

6.4 Report of Equipment Testing

The report shall be issued in draft form for review prior to final publication. The reports shall be prepared by NSF and shall 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.

This report shall be prepared using Microsoft Word® 2003.

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

Appendix A

Method for MS2 Culture, Preparation, and Enumeration (Derived from NSF Standard 55 Annex A)

A.1 Equipment

- autoclave;
- incubator, 35 ± 1 °C (95 ± 1 °F);
- refrigerator, 5 ± 3 °C (41 ± 3 °F);
- water bath 50 ± 1 °C (122 ± 1 °F);
- freezer;
- microwave;
- vortex mixer;
- pH meter;
- Colony Counter; and
- centrifuge.

A.2 Microorganisms

All organisms shall be obtained from ATCC.

- MS2 Coliphage (ATCC # 15597-B1); and
- *Escherichia coli* host strain (ATCC # 15597).

A.3 Supplies

- Petri dishes, 15 x 100 mm: sterile;
- pipettes, 1 mL and 10 mL, sterile;
- sterile centrifuge tubes, 10 mL and 50 mL;
- sample bottles, 125 mL sterile screw cap;
- test tubes, 16 x 125 mm;
- sterile inoculating loop;
- sterile filtration apparatus;
- sterile 0.22 µm polyestersulfone membrane filters; and
- chlorine detection kit.

A.4 Reagents

- Sterile buffered dilution water (SBDW). This shall be prepared according to the *Standard Methods for the Examination of Water and Wastewater* (dilution water: buffered water);

- Phosphate buffer saline (PBS). A stock solution shall be prepared by dissolving 80 g sodium chloride (NaCl), 2 g potassium dihydrogen phosphate (KH_2PO_4), 29 g hydrated

disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$), and 2 g potassium chloride (KCl) in water to a final volume of 1 L. A working solution shall be prepared from the stock solution by diluting 1 volume of the stock with 9 volumes of water. The pH shall be adjusted using a pH meter to 7.4 with 0.1 N HCl or 0.1 N NaOH before use;

– Ethylenediaminetetraacetic acid (EDTA), Sigma # ED2SS; and

– Lysozyme, Boehringer Mannheim, #1 243004. Store at 2 to 8 °C (35 to 46 °F).

A.5 Safety precautions and hazards

A.5.1 Steam sterilized samples and equipment shall be handled with protective gloves when being removed from the autoclave.

A.5.2 Cryogenic culture vials shall be handled with cryoprotective gloves.

A.5.3 All microbiological samples and contaminated test supplies shall be steam sterilized to 121 ± 1 °C (250 ± 1 °F) at 15 psi for a minimum of 20 min prior to being discarded.

A.6 Growth medium

NOTE 1 – Common bacteriological media may be purchased from bacteriological medium manufacturers and prepared according to the manufacturer's instructions.

NOTE 2 – The quality of the growth media shall be monitored by examining growth promotion and sterility prior to use.

A.6.1 TSB (Tryptic Soy Broth)

Ingredient	Amount
tryptone	1.7 g
soytone	0.3 g
dextrose	0.25 g
sodium chloride	0.5 g
dipotassium phosphate	0.25 g
DI water	100 mL
pH	7.3 ± 0.2

TSB shall be dissolved by boiling and adjusted to final pH. 8-mL aliquots shall be dispensed into 16 x 150 mm test tubes. TSB shall be autoclaved at 121 ± 1 °C (250 ± 1 °F) at 15 psi for 20 min. Cooled broth shall be stored at 5 ± 1 °C (41 ± 1 °F).

A.6.2 1.5% TSA (Tryptic Soy Agar)

Ingredient	Amount
tryptone	7.5 g
soytone	2.5 g
sodium chloride	2.5 g
bacto-agar	7.5 g
DI water	500 mL
pH	7.3 ± 0.2

TSA shall be dissolved by boiling, adjusted to final pH, and autoclaved at 121 ± 1 °C (250 ± 1 °F) at 15 psi for 20 min. Tempered media shall be poured into sterile petri dishes. Agar plates shall be stored at 5 ± 1 °C (41 ± 1 °F). Plates shall be allowed to come to room temperature before use.

A.6.3 Phage top agar 1% TSA (Tryptic Soy Agar)

Ingredient	Amount
tryptone	7.5 g
soytone	2.5 g
sodium chloride	2.5 g
agar	5.0 g
DI Water	500 mL
pH	7.3 ± 0.2

TSA shall be dissolved by boiling, adjusted to final pH, and autoclaved at 121 ± 1 °C (250 ± 1 °F) at 15 psi for 20 min. Agar shall be stored at 5 ± 3 °C (41 ± 1 °F). On the day of testing, the TSA shall be liquefied and placed in the 45 ± 1 °C (113 ± 1 °F) water bath. The MS2 Coliphage top agar shall be maintained at 45 ± 1 °C (113 ± 1 °F) to prevent agar solidification.

A.7 Culture of challenge organisms

A.7.1 MS2 Coliphage

A.7.1.1 Stock culture preparation of MS2 Coliphage

NOTE – This section describes the propagation and harvesting methods for stock suspensions of MS2 Coliphage for use as a challenge suspension for low flow (< 1 gpm) water treatment units. If units possessing a flow rate greater than 1 gpm are to be tested, the stock preparation procedure may have to be repeated multiple times to achieve the required volume of MS2 Coliphage. This method should also be repeated when cryogenic stocks are low.

a) One day prior to preparation of MS2 Coliphage stock, a cryogenically frozen *E. coli* host strain shall be thawed. One TSB tube shall be inoculated with 0.1 mL of the stock suspension. The stock suspension shall be incubated at 35 ± 1°C (95 ± 1 °F) for 18 ± 2 h.

b) On the day of preparing MS2 Coliphage stock, 1% TSA shall be liquefied and the media shall be tempered in a 45 ± 1 °C (113 ± 1 °F) water bath. 1.5% TSA plates shall be room temperature prior to use.

c) Serial dilutions of MS2 Coliphage suspension (10^{-1} to 10^{-12}) shall be made using sterile PBS. 10^{-5} to 10^{-12} dilutions shall be plated in triplicate on 1.5% TSA plates. In a sterile tube, 1 mL of diluted MS2 Coliphage shall be transferred. Then 0.1 mL of *E. coli* host shall be added quickly to ~ 5 mL of melted 1% TSA. The inoculum and media shall be vortexed and poured on TSA plates. The plates shall be rocked to spread inoculum evenly. After the 1% TSA layer has solidified, the plates shall be inverted and incubated at 35 ± 1°C (95 ± 1 °F) for 18 ± 2 h.

d) Plates shall be selected that show complete lysis of host cells by the MS2 Coliphage. The surface of each plate shallbe flooded with 3 mL of TSB. The 1% TSA layer shall be gently removed using a cell scraper. The contents shall be poured into two sterile 50 mL centrifuge tubes and the total volume brought to 40 mL with TSB. 0.2 g EDTA and 0.026 g lysozyme shall be added to each tube. The centrifuge tubes shall be incubated at room temperature for 2 h, mixing every 15 min.

e) After the 2 h incubation, the tubes shall be centrifuged at 5251 *xg* for 20 min, at 20 ± 1 °C (68 ± 1 °F). The resulting supernatant shall be removed while avoiding the pellet. A sterile 47-mm filtration assembly shall be aseptically constructed using a 0.22-µm polyestersulfone filter. The filter shall be pretreated with 10 mL of TSB broth just prior to the filtration to minimize MS2 Coliphage adsorption to the filter. The supernatant shall be filtered.

f) For long-term storage (greater than 28 d), $^{1}/_{10}$ volume of sterile glycerol shall be added to suspension, dispensed into 1 mL and 3 mL aliquots in cryovials, and stored at -70° ± 1 °C (-94 ± 1 °F).

g) The MS2 Coliphage suspension shall be titrated as in A.7.1.2. The concentration of MS2 Coliphage should be 10^{10} to 10^{12} PFU/mL.

A.7.1.2 Enumeration of MS2 Coliphage plaques

a) A cryogenically frozen *E. coli* host strain shall be thawed. One TSB tube shall be inoculated with 0.1 mL of the stock suspension. The TSB tube shall be incubated at 35 ± 1 °C (95 ± 1 °F) for 18 ± 2 h.

b) 1% TSA shall be liquefied and the media shall be tempered in a 45 ± 1 °C (113 ± 1 °F) water bath. 1.5% TSA plates shall be room temperature prior to use.

c) Serial dilutions of MS2 Coliphage suspension (10^{-1} to 10^{-12}) shall be made using sterile PBS. 10^{-7} to 10^{-12} dilutions shall be plated in triplicate on 1.5% TSA plates. In a sterile tube, 1 mL of diluted MS2 Coliphage shall be transferred. Then 0.1 mL of *E. coli* host shall be added quickly to ~ 5 mL of melted 1% TSA. The inoculum and media shall be vortexed and poured on TSA plates. The plates shall be rocked to spread inoculum evenly. After the 1% TSA layer has solidified, the plates shall be inverted and incubated at 35 ± 1°C (95 ± 1 °F) for 18 ± 2 h.

d) After incubation, plates containing 20 – 200 distinct plaque forming units (PFU) shall be enumerated using a Colony Counter. The MS2 Coliphage suspension titer shall be calculated by multiplying the number of PFU obtained by the inverse of the dilution factor. The concentration of MS2 Coliphage should be 10^{10} to 10^{12} PFU/mL.

A.8 MS2 challenge suspension preparation

A.8.1 Determination of the concentration of challenge organism

This determination shall be based upon the unit flow rates, injection feed pump rate, suspension density, and the final challenge organism concentration for the unit challenge (typcially $1X10^5$ to $1X10^6$). The suspension shall be of adequate volume to deliver the challenge organism to a minimum of one full test run.

Example:

- unit flow rate: 600 gpm
- test duration: 15 minutes
- injection duration: 15 minutes
- injection rate: 1180 mL/min
- suspension density: 1×10^{10}/mL
- target final concentration: 1.0×10^5/mL

a) To challenge for 15 min at a total of 17.7 L of stock solution is needed to challenge 9000 gallons (34,065 L) of test water:

- $(1.0 \times 10^5/mL)(34{,}065\ L) = (\text{injection feed conc.})(17.7\ L)$; and
- injection feed concentration = 1.92×10^8/mL.

b) To prepare this from the stock suspension, combine:

- $(17.7\ L)(1.92 \times 10^8/mL) = (\text{mL of suspension density})(1.0 \times 10^{10}/mL)$;
- mL of suspension density = 340 mL; and
- 340 mL of suspension to 17,360 mL of PBS.

Once suspension has been made, the suspension shall be mixed using a magnetic stirrer.

A 10-mL aliquot shall be removed from the challenge suspension and set aside for density verification according to *Standard Methods for the Examination of Water and Wastewater.*

A.9 Analysis of feed and filtrate samples

a) Serial dilutions of the influent and effluent samples (10^0 to 10^{-5}) shall be made using sterile PBS. 10^0 to 10^{-5} dilutions shall be plated in duplicate on 1.5% TSA plates. In a sterile tube, 1 mL of diluted MS2 Coliphage shall be transferred. Then 0.1 mL of *E. coli* host shall be added quickly to ~ 5 mL of melted 1% TSA. The inoculum and media shall be vortexed and poured on TSA plates. The plates shall be rocked to spread inoculum evenly. After the 1% TSA layer has solidified, the plates shall be inverted and incubated at 35 ± 1°C (95 ± 1 °F) for 18 ± 2 h.

b) After incubation, plates containing 20 to 200 distinct plaque forming units (PFU) shall be enumerated using a Colony Counter. The MS2 Coliphage suspension titer shall be calculated by multiplying the number of PFU obtained by the inverse of the dilution factor. Results shall be expressed as the number of PFU/mL.