

US EPA ARCHIVE DOCUMENT

CHAPTER 2
EPA/NSF ETV
EQUIPMENT VERIFICATION TESTING PLAN
FOR THE REMOVAL OF MICROBIOLOGICAL AND PARTICULATE CONTAMINANTS
BY MEMBRANE FILTRATION

Prepared by:
NSF International
789 Dixboro Road
Ann Arbor, MI 48105

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1.0 APPLICATION OF THIS EQUIPMENT VERIFICATION TESTING PLAN

This document is the Environmental Technology Verification (ETV) Technology Specific Test Plan (TSTP) for evaluation of water treatment equipment for removal of microbiological and particulate contaminants using membrane filtration. This TSTP is to be used as a guide in the development of Product-Specific Test Plan (PSTP) procedures for testing membrane filtration equipment, within the structure provided by, “*Protocol For Equipment Verification Testing For Physical Removal of Microbiological And Particulate Contaminants: Chapter 1, Requirements For All Studies.*” This TSTP is only applicable to pressure-driven and vacuum-driven membrane processes. It does not apply to:

- Electrically-driven;
- Thermally-driven; or
- Concentration-driven membrane processes.

To participate in the equipment verification process for membrane filtration, the equipment manufacturer and its designated Field Testing Organization (FTO) shall employ the procedures and methods described in this TSTP and in the referenced ETV protocol document as guidelines for the development of the PSTP. The PSTP procedures should generally follow those tasks outlined herein, with changes and modifications made for adaptations to specific membrane equipment. At a minimum, the format of the procedures written in the PSTP for each task should consist of the following sections:

- Introduction;
- Objectives;
- Work Plan;
- Analytical Schedule; and
- Evaluation Criteria.

Each PSTP shall include Tasks 1 to 8. Task 4, maximum pore size reporting and Task 9, raw water pretreatment, are not mandatory. For example, some manufacturers may wish to become verified for raw water pretreatment capabilities. In this case, the components of Task 9 should become a part of the PSTP.

2.0 INTRODUCTION

Pressure-driven membrane processes are currently in use for a broad number of water treatment applications ranging from removal of microbial contaminants such as *Giardia* and *Cryptosporidium*, to removal of natural organic matter contributing to disinfection by-product (DBP) formation. Typically, ultra low pressure membrane processes, such as microfiltration (MF) and ultrafiltration (UF) are employed to provide a physical barrier for removal of microbial and particulate contaminants from drinking waters. Higher pressure membrane applications such as nanofiltration (NF) and reverse osmosis (RO) are typically employed to achieve differing degrees of removal of total organic carbon (TOC), hardness ions, and other inorganic constituents such as salt species, in some applications. Nonetheless, this TSTP is applicable to any pressure-driven or vacuum-driven membrane process.

This TSTP is applicable to any membrane geometry as long as it is adequately described by the manufacturer. Various membrane geometries are currently employed for water treatment applications including:

- Spiral-wound (SW);
- Hollow-fiber (HF);
- Tubular;
- Cassette;
- Cartridge; and
- Flat sheet.

3.0 GENERAL APPROACH

This TSTP is broken down into nine tasks, as shown in the experimental matrix provided in Table 1. As noted above, Tasks 1 to 8 (except 4) shall be performed by any manufacturer wanting the performance of their equipment verified under the ETV Program. Tasks 4 and 9 are optional and can be implemented at the manufacturer’s discretion. The manufacturer’s designated FTO shall provide full detail of the procedures to be followed in each task in the PSTP. The FTO shall specify in the PSTP the operational conditions to be verified during the verification testing. All filtrate flux values shall be reported in terms of temperature-corrected flux values, as either gallons per square foot per day (gfd) at 68°F or liters per square meter per hour (L/(m²-hr)) at 20°C.

Table 1. Task Descriptions			
Task	Testing Periods (minimum)	Issue	Test
Membrane Verification Testing Study			
1 Membrane flux and recovery	1	Rate of specific flux decline	Evaluate productivity at selected set of operational conditions.
2 Cleaning efficiency	1	Cleaning efficiency	Clean system to evaluate flux recovery.
3 Finished water quality	1	Finished water quality and rejection capabilities	Measure water quality & rejection capabilities.
4 Maximum pore size reporting	optional	Reporting of 90% and maximum pore size	Report 90% and maximum pore size for the membrane tested.
5 Membrane Integrity Testing	1	Integrity of membrane surface	Investigate integrity of membrane surface.
6 Data handling protocol			
7 QA/QC			
8 Microbial Removal	1	Removal of protozoa, bacteria, virus or surrogates	Conduct seeding experiments using MS2 virus, <i>Giardia</i> , <i>Cryptosporidium</i> , and/or surrogates if there is a relationship between the surrogate and the target microorganism that has been proven by peer-reviewed studies and proven methodologies.
9 Raw water pretreatment	optional	Pretreatment techniques that are not considered necessary	Demonstrate membrane performance after pretreatment and determine efficacy of pretreatment.

The total verification testing plan shall be performed over a one-month period (not including time for system set-up, shakedown and mobilization). At a minimum, one one-month period of verification testing shall be conducted to provide equipment testing information.

4.0 OVERVIEW OF TASKS

This section provides a brief overview of the required and optional tasks included in this TSTP.

4.1 Task A: Characterization of Feed Water

The objective of this initial operations task is to obtain a chemical, biological, and physical characterization of the feed water prior to testing.

4.2 Task B: Initial Test Runs

The objective of this initial operations task is to evaluate equipment operation and determine the treatment conditions that result in effective treatment of the feed water. This task is considered shakedown testing and shall be carried out prior to performing Tasks 1 through 8 (and Task 9, if applicable).

4.3 Task 1: Membrane Flux and Recovery

Task 1 will evaluate membrane operation and will entail quantification of membrane flux decline rates and product water recoveries. The rates of flux decline will be used to demonstrate membrane performance at the specific operating conditions to be verified. The specific operating conditions to be verified are the treatment conditions established during Task B initial test runs.

4.4 Task 2: Cleaning Efficiency

An important aspect of membrane operation is the restoration of membrane productivity after membrane flux decline has occurred. The objective of this task is to evaluate the efficiency of the membrane cleaning procedures recommended by the manufacturers. The fraction of specific flux that is restored following a chemical cleaning and after successive filter runs will be determined.

4.5 Task 3: Finished Water Quality

The objective of this task is to evaluate the quality of water produced by the membrane system. Multiple water quality parameters will be monitored during each test period. The mandatory water quality monitoring parameters shall include: turbidity, particle concentrations, total suspended solids (TSS), TOC, UV absorbance (at 254 nm wavelength), coliforms, and heterotrophic plate count (HPC) bacteria populations. Other water quality parameters will be optional, such as DBP formation potential. A basic goal of this task is to confirm that membrane treated waters meet manufacturer's stated performance capabilities. Water quality produced will be evaluated in relation to feed water quality and operational conditions.

4.6 Task 4: Reporting of Membrane Pore Size (Optional)

Membranes for particle and microbial removal do not have a single pore size, but rather have a distribution of pore sizes. For example, a nominally rated 0.1 μm MF membrane may have pores ranging from 0.08 μm to 0.4 μm . Membrane rejection capabilities are thus limited by the maximum membrane pore size. The objective of this task is to report the 90% and maximum membrane pore size of the membranes employed in field operations. This is a suggested task.

4.7 Task 5: Membrane Module Integrity

A critical aspect of any membrane process is the ability to verify that a membrane process is producing a specified water quality on a continual basis. For example, it is important to know whether the membrane is providing a constant barrier to protozoan oocysts such as *Cryptosporidium*. The objective of this task is to demonstrate the methodology to be employed for monitoring membrane integrity and to verify the integrity of membrane modules.

4.8 Task 6: Data Handling Protocol

The objective of this task is to establish an effective field protocol for data management at the field operations site and for data transmission between the FTO and NSF International (NSF).

4.9 Task 7: Quality Assurance and Quality Control

An important aspect of verification testing is the protocol developed for quality assurance and quality control (QA/QC). The objective of this task is to assure accurate measurement of operational and water quality parameters during membrane equipment verification testing.

4.10 Task 8: Microbial Removal

The objective of this task is to evaluate microbial removal capabilities by seeding the systems with target organisms which shall include, but are not limited to, selected protozoa and viruses. The manufacturer shall choose to have either a field microbial seeding study or bench-scale microbial testing performed as part of their verification testing. The introduction of surrogates for protozoa and viruses may be allowed only when peer-reviewed studies and proven methodologies have shown the relationship between surrogates and target microorganisms.

4.11 Task 9: Raw Water Pretreatment (Optional)

Most membrane processes that are employed for particle and microbial removal require no pretreatment, except for pre-screening, and therefore require no optional pretreatment testing per the requirements of this TSTP. Furthermore, in cases where a pretreatment technique is considered an integral part or inseparable part of the function of the membrane system, no additional testing of system pretreatment capabilities would be necessary. However, some manufacturers may wish to employ an optional pretreatment technique that does not represent an integral part of the membrane technology for removal of microbiological and particulate contaminants. Such optional pretreatment may be employed to extend membrane operational time or remove selected contaminants.

The objective of this raw water pretreatment task is to evaluate the efficacy of raw water pretreatment for improvement of membrane operation or removal of selected contaminants. The specific goals of this task will be to evaluate raw water pretreatment required prior to membrane filtration and to evaluate any changes in treated water quality associated with raw water pretreatment.

5.0 TESTING PERIODS

The required tasks of the TSTP (Tasks 1 through 8, except Task 4) are designed to be completed over a minimum of one verification testing period of one month (30 days), not including mobilization, shakedown and start-up. Membrane testing conducted beyond the testing period may be used for fine-tuning of membrane performance or for evaluation of additional operational conditions. Many of the tasks presented as Tasks 2 through 7 can be performed concurrent with Task 1, the flux and operational testing procedures. Task 8 may also be conducted during the testing period if a field study is chosen or before, during or after the testing period, if a bench-scale laboratory test is chosen. However, Task 9 shall be performed in an additional month of testing.

Additional verification testing periods may be necessary to verify the manufacturer's statement of performance capabilities, such as in the treatment of surface water where additional testing during each season may assist in verifying a statement of performance capability. For systems treating solely groundwater or surface waters of consistent quality due to pre-treatment, one verification testing period may be sufficient. If one verification testing period is selected, the feed water should represent the worst-case concentrations of contaminants which can verify the manufacturer's statement of performance capabilities. For example, a good challenge for a membrane would be a test period during which the feed water exhibits low temperature, high turbidity and/or natural organic matter. Although one test period satisfies the minimum requirement of this TSTP, manufacturers are encouraged to use additional testing periods to cover a wider range of water quality conditions.

Verification testing periods consist of continued evaluation of the treatment system using the pertinent treatment parameters defined in initial operations. Performance and reliability of the equipment shall be tested during verification testing periods at a minimum of 30 days. The purpose of the one month test period is to demonstrate the ability of the equipment to meet the water quality goals specified by the manufacturer, the product water recovery, and the rate of flux decline observed over the one month period of operation.

6.0 DEFINITION OF OPERATIONAL PARAMETERS

Definitions that may apply to membrane filtration include:

- 6.1 **Filtrate:** Water produced by the membrane filtration process.
- 6.2 **Feed Water:** Water introduced to the membrane module.
- 6.3 **Filtrate Flux:** The average filtrate flux is the flow of product water divided by the surface area of the membrane. It should be noted that gfd and L/(m²-hr) shall only be used as units of flux. Filtrate flux is calculated according to the following formula:

$$J_t = \frac{Q_p}{S}$$

where J_t = filtrate flux at time t (gfd, L/(m²-hr);
 Q_p = filtrate flow (gpd, L/h); and
 S = membrane surface area (ft², m²).

- 6.4 Specific Flux:** The term specific flux is used to refer to filtrate flux that has been normalized for the transmembrane pressure. The equation used for calculation of specific flux is given as follows:

$$J_{tm} = \frac{J_t}{NDP}$$

where J_{tm} = specific flux at time t (gfd/psi, L/(m²-hr)/bar);
 J_t = filtrate flux at time t (gfd, L/(m²-hr)); and
 P_{tm} = transmembrane pressure (psi, bar).

Specific flux results shall always be reported with indication of the time interval after initiation of the experimental test run.

- 6.5 Membrane Fouling:** A reduction in filtrate flux that can be restored by mechanical or chemical means is termed “reversible” fouling. In contrast, “irreversible fouling” is defined as a permanent loss in filtrate flux capacity that cannot be restored. The fouling of membranes designed for particle or microbial removal is primarily attributed to deposition of materials on the membrane surface and/or in the membrane pores.

- 6.6 Transmembrane Pressure:** The average transmembrane pressure is calculated:

$$P_{tm} = \left[\frac{(P_i + P_o)}{2} - P_p \right]$$

where P_{tm} = transmembrane pressure (psi, bar);
 P_i = pressure at the inlet of the membrane module (psi, bar);
 P_o = pressure at the outlet of the membrane module (psi, bar); and
 P_p = filtrate pressure (psi, bar).

- 6.7 Temperature Adjustment for Flux Calculation:** Temperature corrections to 20°C for transmembrane flux shall be made to correct for the variation of water viscosity with temperature. A specific, empirically derived equation developed by the membrane manufacturer may be used to provide temperature corrections. Alternatively, the following equation by Streeter and Wiley (1985) may be employed:

$$J_t \text{ (at } 20^\circ \text{ C)} = \frac{Q_p \times e^{-0.0239(T-20)}}{S}$$

where J_t = instantaneous flux (gfd, L/(m²-hr));
 Q_p = filtrate flow (gpd, L/h);
 T = temperature, (°F, °C); and
 S = membrane surface area (ft², m²).

6.8 Feed Water System Recovery: The recovery of filtrate from feed water is given as the ratio of filtrate flow to feed water flow:

$$\% \text{ System Recovery} = 100 \cdot \left[\frac{Q_p}{Q_f} \right]$$

where Q_p = filtrate flow (gpd, L/h) and
 Q_f = feed flow to the membrane (gpd, L/h).

6.9 Membrane Element Recovery: The recovery of filtrate from total recirculation influent water is given as the ratio of filtrate flow to the sum of feed water flow and recycle flow:

$$\% \text{ Element Recovery} = 100 \left[\frac{Q_p}{Q_f + Q_r} \right]$$

where Q_p = filtrate flow (gpd, L/h);
 Q_f = feed flow to the membrane (gpd, L/h); and
 Q_r = recycle flow (gpd, L/h).

7.0 TASK A: CHARACTERIZATION OF FEED WATER

This initial operations task is needed to determine if the chemical, biological, and physical characteristics of the feed water are appropriate for the water treatment equipment to be tested.

7.1 Objectives

The objective of this task is to obtain a complete chemical, biological, and physical characterization of the feed water that will be entering the treatment system being tested.

7.2 Work Plan

This task can be accomplished by using analytical measurements obtained from third party sources (i.e. USGS, EPA, state laboratories, municipal laboratories). The specific parameters needed to characterize the water will depend on the equipment being tested but information on the following characteristics should be compiled:

- Temperature, pH, turbidity, and UV₂₅₄ absorbance;
- Total alkalinity and total hardness;
- TOC, Total Dissolved Solids (TDS), and TSS; and
- Total coliform (TC) and HPC bacteria.

If sufficient historic data is not available to properly evaluate the feed water quality, additional monitoring of the feed water should be performed to adequately assess feed water quality. Ideally, one year of historic water quality data for each parameter will be available for the proposed feed water. At a minimum, one month of data, sampled at no greater than weekly intervals, may constitute historic data.

Sufficient information shall be obtained to illustrate the variations expected to occur in these parameters that will be measured during verification testing for the water source. This information shall be compiled and shared with NSF, so NSF and the FTO can determine the adequacy of the data for use as the basis to make decisions on the testing schedule. Failure to adequately characterize the feed water could result in testing at a site later deemed inappropriate, so the initial characterization will be important to the success of the testing program.

7.3 Analytical Schedule

In many cases, sufficient water quality data may already exist to permit making a determination of the suitability of a source water for use as feed water in a membrane verification testing program. If sufficient historic data is not available to properly evaluate the source water quality, additional monitoring of the source water shall be performed to adequately assess source water quality.

7.4 Evaluation Criteria

Feed water quality will be evaluated in the context of the manufacturer's statement of performance capabilities. The feed water should challenge the capabilities of the equipment but should not be beyond the range of water quality suitable for treatment for the equipment in question.

8.0 TASK B: INITIAL TEST RUNS

8.1 Objectives

The objective of initial test runs, also called shakedown testing, is to evaluate equipment operation and determine the treatment conditions that result in effective treatment of the feed water.

8.2 Work Plan

Initial test runs shall be conducted so a preliminary assessment of treatment performance can be made. If more than one verification test period is planned, this task shall occur prior to each test period. This task is considered shakedown testing and shall be carried out prior to performing Tasks 1 through 9 (Tasks 4 and 9 are optional).

8.3 Analytical Schedule

Because these runs are being conducted to determine the suitability of the technology for verification testing, a strictly defined schedule for sampling and analysis does not need to be followed. Adhering to the schedule for sampling and analysis to be followed during verification testing would be wise so the operator can gain familiarity with the time requirements that will be applicable later on in the test program.

8.5 Evaluation Criteria

The manufacturer and FTO shall evaluate the data produced during the initial test runs to determine if the water treatment equipment performance met or exceeded expectations based on the statement of performance capabilities. If the performance was not as good as the statement of performance capabilities, the manufacturer may wish to conduct more initial test runs or to cancel the testing program.

9.0 TASK 1: MEMBRANE FLUX AND OPERATION

9.1 Introduction

Membrane operation will be evaluated in this task, with quantification of membrane flux decline rates and product water recoveries. The rates of flux decline will be used to demonstrate membrane performance at the specific operating conditions to be verified. The operational conditions to be verified shall be specified by the manufacturer and described by the FTO in the PSTP in terms of a temperature-corrected flux value (e.g., gfd at 68°F or L/(m²-hr) at 20°C) before the initiation of the verification test.

The rate of specific flux decline is a function of water quality and operational conditions. In this task, water quality shall be monitored and operational conditions varied depending upon membrane flux decline profiles. Flow and pressure data shall be collected to quantify the loss of productivity in terms of rate of specific flux decline. A lower rate of specific flux decline implies that a longer operational run will be achieved by the membrane system.

9.2 Experimental Objectives

The objectives of this task are to demonstrate: 1) the appropriate operational conditions for the membrane equipment; 2) the product water recovery achieved by the membrane equipment; and 3) the rate of flux decline observed over extended membrane filtration operation. Raw water quality shall be monitored (Task 3) during each seasonal one-month testing period at a minimum, to track any significant variations that could impact rates of membrane flux decline.

It should be noted that the objective of this task is not process optimization, but rather verification of membrane operation at the operating conditions specified by the manufacturer and described by the FTO in the PSTP, as pertains to filtrate flux and transmembrane pressure.

9.3 Work Plan

Determination of optimal membrane operating conditions for a particular water can typically require as long as one year of operation. For this task, the manufacturer shall specify the operating conditions and shall supply written procedures on the operation and maintenance (O&M) of the membrane treatment system. The manufacturer shall also specify the termination criteria for their particular membrane equipment. For example, the termination criteria may consist of an 80% decline in specific flux, or increase in transmembrane pressure to a specific value. In this task, each set of operating conditions shall be maintained for the one month testing period (continuous 24-hour operation). The manufacturer shall specify the primary filtrate flux at which the equipment is to be

verified. The FTO shall describe the operating conditions and include a copy of the manufacturer's O&M manual in the PSTP.

After set-up and shakedown of membrane equipment, membrane operation should be established at the flux condition to be verified. The membrane system shall be operated as shown schematically in Figure 1 for a minimum of one month. If substantial specific flux decline of the membrane occurs at the specified flux before the one month operating period is complete, chemical cleaning shall be performed and adjustments to the operational strategy shall be made (such as a decrease in transmembrane flux or an increase in backwash frequency, if applicable). The manufacturer shall make decisions on adjustments to the operational strategy. At a minimum, the membrane shall be chemically cleaned according to the manufacturer specifications at the conclusion of the one month period. At this time, the cleaning efficiency will be determined per Task 2.

This membrane TSTP has been written with the aim to balance the costs of verification with the benefits of testing membrane filtration over a wide range of operating conditions. Given that it may take as long as a month and longer to observe significant flux decline in a membrane system, examination under a wide range of operating conditions would be prohibitively expensive for the membrane manufacturer. Therefore, this TSTP requires that one set of operating conditions be tested for a one-month testing period. It shall be furthermore understood that beyond the single set of verification operating conditions, membrane operation that occurs at a lower flux, a lower recovery, or a higher cross-flow velocity shall also constitute a verifiable condition.

To establish appropriate conditions of flux, recovery, backwash frequency and duration, the manufacturer may have some experience with his equipment on a similar water source. This may not be the case for suppliers with new products. In this case, it is advisable to perform shakedown tests as described in Task B so that reasonable operating criteria can be established. This would aid in preventing the unintentional but unavoidable optimization during the verification testing.

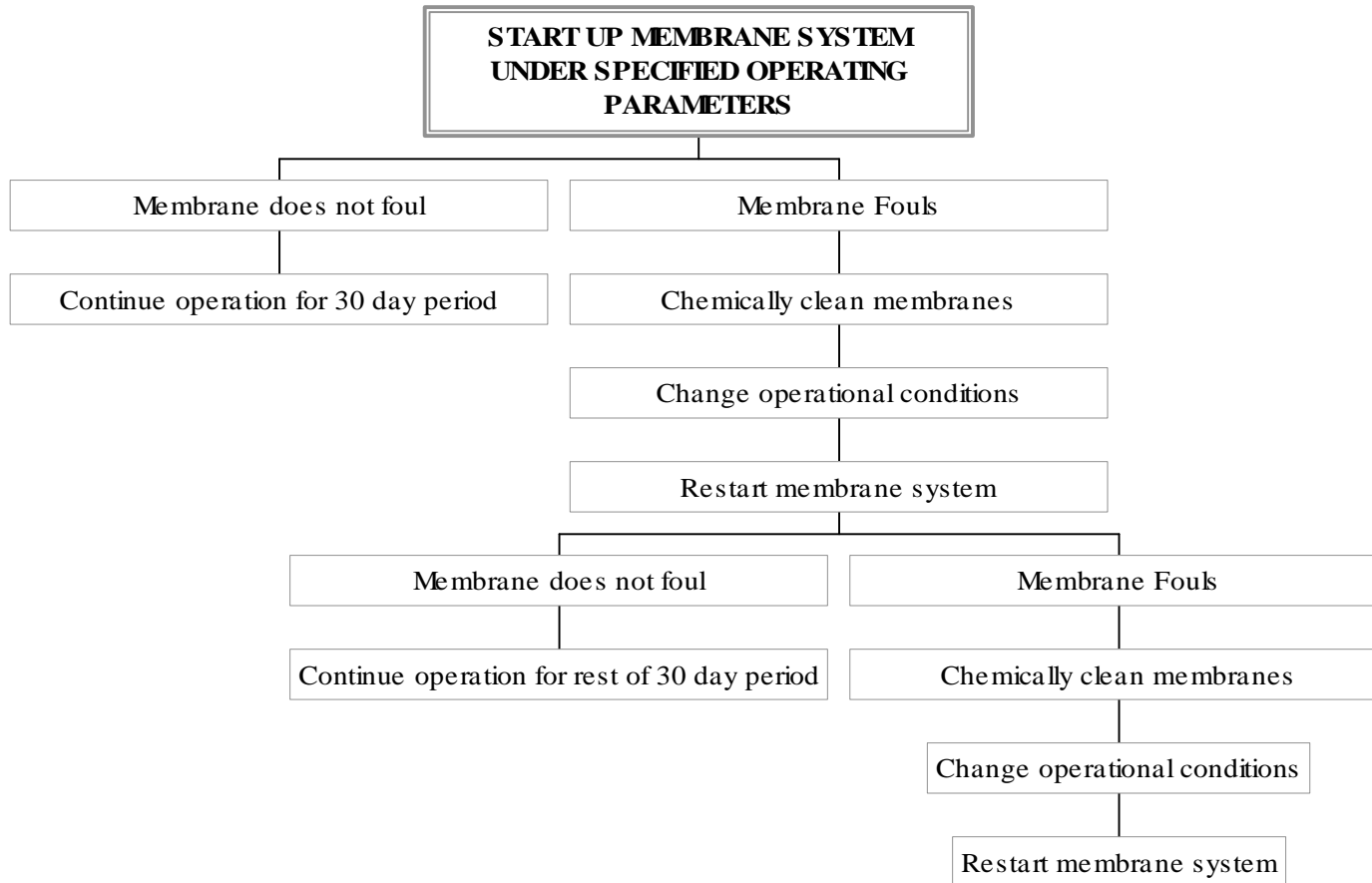
Testing of additional operational conditions may be included in the verification testing program at the discretion of the manufacturer and its designated FTO. However, testing of alternate additional operational conditions shall be performed by including additional one-month testing periods beyond the one month required by the TSTP.

Additional months of testing may also be included in the PSTP to demonstrate membrane performance under different feed water quality conditions. For membrane filtration, extremes of feed water quality (e.g., low temperature, high turbidity) are the conditions under which membranes are most prone to rapid flux decline and to failure. The FTO shall perform testing with as many different water quality conditions as desired for verification status. Testing under each different water quality condition shall be performed during an additional one-month testing period, as required above for each additional set of operating conditions.

The testing runs conducted under this task shall be performed in conjunction with Tasks 2, 3, 5, 8 (if a field seeding study is conducted) and the optional Task 9. With the exception of the additional testing periods conducted at the manufacturer and FTO's discretion, no additional membrane test runs are required for performance of Tasks 2, 3, 5, 8 or 9.

Figure 1

Schematic of Membrane Operational Plan



9.4 Analytical Schedule

9.4.1 Operational Data Collection

Measurement of membrane feed water flow and filtrate flow (recycle flow where applicable), system pressures and feed water temperature shall be collected at a minimum of two times per day. Table 2 presents the operational data collection schedule. Measurement of feed water temperature to the membrane shall be made daily to provide data for correction of transmembrane flux.

Location	Minimum Frequency
Raw	
Flow	2/day
Feed water Temperature	1/day
Single Stage Membrane Processes	
Influent module/vessel pressure	2/day
Effluent module/vessel pressure	2/day
Filtrate pressure	2/day
Filtrate flow	2/day
Multiple Stage Membrane Processes	
Stage 1 Influent module pressure	2/day
Stage 1 Effluent module pressure	2/day
Stage 1 Feed flow	2/day
Stage 1 Filtrate pressure	2/day
Stage 1 Filtrate flow	2/day
Stage 2 Influent module pressure	2/day
Stage 2 Effluent module pressure	2/day
Stage 2 Feed flow	2/day
Stage 2 Effluent module flow	2/day
Stage 2 Filtrate pressure	2/day
Stage 2 Filtrate flow	2/day
Crossflow velocity	2/day

Note: The FTO should adapt the operational data collection location to the particular geometry of the membrane system.

In an attempt to calculate cost factors for small-scale operation of membrane equipment, power usage for operation of the membrane equipment shall also be closely monitored and recorded by the FTO during each testing period. Power usage shall be estimated by inclusion of the following details regarding equipment operation requirements: (pumping requirements, size of pumps, nameplate voltage, current draw, power factor, chemical usage, etc.). In addition, measurement of power consumed shall be provided by information on current draw and power consumption. Chemical usage shall be quantified by recording day tank concentration and daily volume consumption. No additional operational data shall be required by Tasks 2 and 3 unless specifically stated.

9.4.2 Feed Water Quality Limitations

The characteristics of feed waters used during the testing period (and any additional one-month testing periods) shall be explicitly stated in reporting the membrane flux and recovery data for each season. Accurate reporting of such feed water characteristics as temperature, turbidity, and TSS is critical, as these parameters may substantially influence the range of achievable membrane performance on a seasonal basis. In addition, accurate reporting of water quality characteristics such as pH, alkalinity, and TOC shall be reported on a monthly basis to provide a general background on the source water character and quality for each testing period. More frequent monitoring of these parameters may be performed if desired by the manufacturer or recommended by FTO.

9.5 Evaluation Criteria and Minimum Reporting Requirements

- Transmembrane pressure (P_{tm}) :
 - Plot graph of transmembrane pressure over time for each 30 day period of operation.
- Rate of specific flux decline:
 - Plot graph of specific flux normalized to 20°C over time for each 30 day period of operation.
- Cleaning efficiency:
 - Provide table of intervals between chemical cleaning episodes and efficiency of cleaning achieved following each 30 day period of operation.

10.0 TASK 2: CLEANING EFFICIENCY

10.1 Introduction

Following the test runs of Task 1, the membrane equipment may require chemical cleaning to restore membrane productivity. The number of cleaning efficiency evaluations shall be determined by the rate of specific flux decline of the membrane during the test period. At a minimum, one cleaning shall be performed at the conclusion of the required testing. In the case where the membrane does not fully reach the operational criteria for termination as specified by the manufacturer and its designated FTO in Task 1, chemical cleaning shall be performed after the 30 days of operation, with a record made of the operational conditions before and after cleaning.

10.2 Experimental Objectives

The objective of this task is to evaluate the effectiveness of chemical cleaning for restoring finished water productivity to the membrane systems. The intent of this task is to confirm that standard manufacturer-recommended cleaning practices are sufficient to restore membrane productivity for the systems under consideration. Cleaning chemicals and cleaning routines shall be based on the recommendations of the manufacturer; this task is considered a “proof of concept” effort, not an optimization effort. It should be noted that cleaning solution selection is typically feed water quality specific. The PSTP procedures should permit evaluation of cleaning solutions that are considered optimal for water being treated. If the manufacturer determines that a pre-selected cleaning formulation is not effective, the PSTP procedures should allow the manufacturer to modify it.

10.3 Work Plan

The membrane systems may experience substantial specific flux decline during the membrane test runs conducted for Task 1. At the conclusion of the test period, membranes shall be utilized for the cleaning assessments herein. No additional experiments shall be required to produce specific flux decline such that chemical cleaning evaluations be performed. Each system shall be chemically cleaned using the recommended cleaning solutions and procedures specified by the manufacturer. After each chemical cleaning of the membranes, the system shall be restarted and the initial conditions of specific flux recovery and rejection capabilities shall be tested.

The manufacturer and its designated FTO shall specify in detail the procedure(s) for chemical cleaning of the membranes in the PSTP. At a minimum, the following shall be specified:

- Cleaning chemicals;
- Quantities cleaning chemicals;
- Hydraulic conditions of cleaning;
- Duration of each cleaning step;
- Initial and final temperatures of chemical cleaning solution; and
- Quantity and characteristics of residual waste volume to be disposed.

In addition, detailed procedures describing the methods for pH neutralization of the acid or alkaline cleaning solutions should be provided along with information on the proper disposal method for regulated chemicals. A description of all cleaning equipment and its operation shall be included in the PSTP.

10.4 Analytical Schedule

10.4.1 Sampling

The pH, turbidity and TDS of each cleaning solution shall be determined and recorded during various periods of the chemical cleaning procedure. In addition, in the case that the cleaning solution employs an oxidant, such as chlorine, the concentration of the oxidant both before and at the end of the cleaning should be measured. Notes recording the visual observations (color, degree of suspended matter present) shall also be provided by the FTO. No other water quality sampling shall be required.

10.4.2 Operational Data Collection

Flow, pressure, and temperature data shall be collected during the cleaning procedure if possible and shall be recorded immediately preceding system shutdown due to substantial membrane flux decline; flow, pressure, and temperature data shall also be collected immediately upon return to membrane operation, after chemical cleaning.

10.5 Evaluation Criteria and Minimum Reporting Requirements

At the conclusion of each chemical cleaning event and upon return to membrane operation, the initial condition of transmembrane pressure, recovery and temperature shall be recorded and the specific flux calculated. The efficacy of chemical cleaning shall be evaluated by the recovery of specific flux after chemical cleaning as noted below, with comparison drawn from the cleaning efficacy achieved during previous cleaning evaluations. Comparison between chemical cleanings shall allow evaluation of the potential for irreversible loss of specific flux and projections for usable membrane life.

Two primary indicators of cleaning efficiency and restoration of membrane productivity will be examined in this task:

1) The immediate recovery of membrane productivity, as expressed by the ratio between the final specific flux value of the current filtration run (J_{s_f}) and the initial specific flux (J_{s_i}) measured for the subsequent filtration run:

$$\% \text{ Recovery of Specific Flux} = 100 \left[1 - \frac{J_{s_f}}{J_{s_i}} \right]$$

where: J_{s_f} = Specific flux (gfd/psi, L/(m²-hr)/bar) at end of current run (final) and
 J_{s_i} = Specific flux (gfd/psi, L/(m²-hr)/bar) at beginning of subsequent run (initial).

2) The loss of specific flux capabilities, as expressed by the ratio between the initial specific flux for any given filtration run (J_{s_i}) divided by the specific flux ($J_{s_{io}}$) at time zero, as measured at the initiation of the first filtration run in a series:

$$\text{Loss of Original Specific Flux} = 100 \left[1 - \frac{J_{s_i}}{J_{s_{io}}} \right]$$

where: $J_{s_{io}}$ = Specific flux (gfd/psi, L/(m²-hr)/bar) at time zero point of membrane testing.

The minimum reporting requirements shall include presentation of the following results:

- Flux recovery:
 - Provide table of post cleaning flux recoveries during each 30 day period of operation.
- Cleaning efficacy:
 - Provide table of cleaning efficacy indicators described above for chemical cleaning procedures performed during each 30 day period of operation.
- Assessment of irreversible loss of specific flux and estimation of usable membrane life for costing purposes.

11.0 TASK 3: FINISHED WATER QUALITY

11.1 Introduction

Water quality data shall be collected for the feed water and membrane filtrate water as shown in the sampling schedule Table 3, during the membrane test runs of Task 1. At a minimum, the required sampling schedule shown in Table 3 shall be observed by the FTO. Water quality goals and target removal goals for the membrane equipment shall be recorded in the PSTP.

11.2 Experimental Objectives

The objective of this task is to assess the ability of the membrane equipment to meet the water quality goals specified by the manufacturer. A list of the minimum number of water quality parameters to be monitored during equipment verification testing is provided in the analytical schedule section below and in Table 3. The actual water quality parameters selected for testing shall be stipulated by the FTO in the PSTP.

11.3 Work Plan

Many of the water quality parameters described in this task shall be measured on-site by the FTO (refer to Table 4). Analysis of the remaining water quality parameters shall be performed by a laboratory that is certified, accredited or approved by a state, a third-party organization (i.e., NSF), or the EPA. The methods to be used for measurement of water quality parameters in the field are described in the analytical methods section below and in Table 4. The analytical methods utilized in this study for on-site monitoring of feed water and filtrate water qualities are described in Task 7, QA/QC. Where appropriate, the *Standard Methods* reference numbers and EPA method numbers for water quality parameters are provided for both the field and laboratory analytical procedures.

For the water quality parameters requiring analysis at a laboratory, water samples shall be collected in appropriate containers (containing preservatives as applicable) prepared by the laboratory. These samples shall be preserved, stored, shipped and analyzed in accordance with appropriate procedures and holding times, as specified by the laboratory.

11.4 Analytical Schedule

11.4.1 Feed and Filtrate Water Characterization

At the beginning of the testing period at a single set of operating conditions (and thereafter with indicated frequency), the raw water and filtrate water shall be characterized by measurement of the following water quality parameters (as indicated in Table 3):

- Alkalinity and Hardness (both monthly);
- TSS and TDS (both once every two weeks);
- TOC and UV_{254 nm} absorbance (both monthly);
- TC and HPC bacteria (once per week);
- Temperature (daily, feed only);
- pH (twice per week);
- Filtrate water turbidity and particle concentrations (twice daily); and
- Feed (and concentrate) water turbidity and particle concentrations (twice daily).

Table 3. Water Quality Sample Schedule

Parameter	Sampling Frequency	Single Stage Process			Multiple Stage Processes				
		Feed	Filtrate	Back-wash Waste	Stage 1 Feed	Stage 1 Filtrate	Stage 1 Concentrate	Stage 2 Filtrate	Stage 2 Backwash Waste
On-Site Analytes									
pH	Twice/week	1	0	0	1	1	1	1	1
Temperature	Daily	1	0	0	1	0	0	0	0
Turbidity	Daily	2	C ¹	2	2	C ¹	2	C ¹	2
Particle counts	Daily	2	C ¹	0	2	C ¹	1	C ¹	1
Laboratory Analysis									
Alkalinity	Monthly	1	1	0	1	1	1	1	1
Total/calcium hardness	Monthly	1	1	0	1	1	1	1	1
TDS	Once/2 weeks	1	1	0	1	1	1	1	1
TSS	Once/2 weeks	1	1	1	1	1	1	1	1
TC	Weekly	1	1	1	1	1	1	1	1
HPC	Weekly	1	1	0	1	1	1	1	1
TOC	Monthly*	1	1	0	1	1	1	1	1
UVA	Monthly*	1	1	0	1	1	1	1	1
DBP Formation Potential Analysis(Optional)									
Total THMs	Monthly	1	1	0	1	1	0	1	0
HAA6	Monthly	1	1	0	1	1	0	1	0
C ¹ continuous monitoring * more frequent monitoring may be performed at the discretion of the manufacturer or FTO.									

Table 4. Analytical Methods

Parameter	Facility	Standard Methods ¹ number or Other Method Reference	EPA Method ²
General Water Quality			
Temperature	On-Site	2550 B	
pH	On-Site	4500-H ⁺ B	150.1 / 150.2
Total alkalinity	Lab	2320 B	
Total Hardness	Lab	2340 C	
Calcium Hardness	Lab	3500-Ca D	
TSS	Lab	2540 D	
TDS	Lab	2540 C	
Particle Characterization			
Turbidity Bench top	On-Site	2130 B / Method 2	180.1
Turbidity In Line	On-Site	Manufacturer	
Particle Counts Bench top	On-Site	Manufacturer	
Particle Counts In Line	On-Site	Manufacturer	
Organic Compound Characterization			
TOC/DOC	Lab	5310B/5310C	
UV ₂₅₄ absorbance	Lab	5910 B	
Total THMs	Lab		524.2; 502.2
HAA6	Lab	6251B	552.1
Microbiological			
TC and HPC	Lab	9221 / 9222 / 9223 / 9215 B	
<i>Cryptosporidium</i>	Lab	NSF and EPA may consider alternative methods if sufficient data on precision, accuracy, and comparative studies are available for alternative methods.	EPA 1622, EPA 1623
MS2 virus	Lab		EPA ICR Method for Coliphage Assay, 1996
Notes: 1) <i>Standard Methods</i> Source: 20th Edition of Standard Methods for the Examination of Water and Wastewater, 1999, American Water Works Association. 2) EPA Methods Source: EPA Office of Ground Water and Drinking Water. EPA Methods are available from the National Technical Information Service (NTIS).			

11.4.2 Water Quality Sample Collection

Water quality data shall be collected at regular intervals during the period of membrane testing, as required in Table 3. For verification of particulate removal, turbidity and particle concentrations in filtrate waters shall be monitored continuously using either batch or in-line analytical instruments. Grab samples of feed waters to the membrane system shall be measured by the FTO twice daily for turbidity and particle concentrations using bench-top analytical equipment. The specific particle size ranges to be monitored by both in-line and bench-top analytical equipment during the verification testing are indicated in Task 7, the QA/QC section.

Water quality parameters including pH and temperature shall be monitored daily. TSS shall be monitored every other week and results of this analysis will be used to construct a mass balance of suspended solids through the membrane system. Monitoring of organic water quality parameters such as TOC and UV₂₅₄ absorbance shall be performed on a monthly basis to evaluate rejection of organics by the membrane. Additional sampling and data collection may be performed at the discretion of the FTO.

In the case of water quality samples to be shipped to the laboratory that is certified, accredited or approved by a state, a third-party organization (i.e., NSF), or the U.S. EPA for analysis, the samples shall be collected in appropriate containers (containing preservatives as applicable) prepared by the laboratory. These samples shall be preserved, stored, shipped, and analyzed in accordance with appropriate procedures and holding times, as specified by the analytical laboratory. All PSTPs shall include, at a minimum, a table(s) showing the parameters to be analyzed, analytical method, the laboratory reporting limit or quantitation limit, sample volume, bottle type, preservation method, and holding time.

On a weekly basis, samples of raw and filtrate waters shall be collected for analysis of indigenous bacterial densities including: TC and HPC. Collected samples shall be placed in a cooler with blue ice to be shipped with an internal cooler temperature of approximately 2-8°C to a laboratory that is certified, accredited or approved by a state, a third-party organization (i.e., NSF), or the EPA. Samples shall be processed for analysis by the laboratory within 24 hours of collection. The laboratory shall then keep the samples at a temperature of approximately 2-8°C until initiation of analysis. TC densities will be reported as most probable number per 100 mL (MPN/100 mL) and HPC densities will be reported as colony forming units per milliliter (cfu/mL).

11.4.3 Feed Water Quality Limitations

The characteristics of feed waters encountered during the testing period shall be explicitly stated in reporting the membrane flux and recovery data. Accurate reporting of such feed water characteristics as temperature, turbidity, TSS, pH, alkalinity and hardness is critical for the verification testing program, as these parameters can substantially influence membrane performance on a seasonal basis.

11.4.4 Turbidity Spiking (Optional Task)

If the anticipated turbidity at the selected site does not challenge the system to the limits of its performance capabilities, an optional turbidity augmentation procedure may be implemented after the 30 days of verification testing has been completed. A procedure for turbidity spiking was published in *Journal American Water Works Association* (AWWA) in December 1993, pp. 39-46 by Logsdon et al. A spiking procedure based on the published technique is described in the following paragraphs. (In this ETV document, when the word “tank” is used, this term includes a storage tank, an above-ground swimming pool of appropriate size, an earthen basin having a plastic liner, or any other device or means of holding large volumes of water.)

To spike turbidity, use of a local turbidity source is recommended. This could consist of sediments taken from the bottom of a river or lake, or natural soil of the type likely to erode into nearby watercourses and cause turbid waters. For testing done in many locations in the United States where row crop agriculture is practiced, topsoil could be used to prepare a suspension for turbidity spiking, because topsoil is a major contributor to turbid runoff as a result of heavy rains in such locations. Topsoil or sediments would be expected to contain some natural organic matter, and as such would enable the FTO to produce a turbidity suspension typical for much of the turbid runoff found in the United States.

The soil or sediments that will be used to prepare a suspension for turbidity spiking should be screened through a three inch screen to remove rocks, for protection of pumps that will be used to mix soil and water.

After screening, soil or sediment should be added in a batch tank having a capacity in the range of 400 to 1000 gallons. Mixing can be accomplished by using a pump with a flow capacity, expressed in gallons per minute, of about 10% of the batch tank volume, expressed in gallons. For a 400 gallon batch tank, a 40 gpm pump theoretically could pump one tank volume in ten minutes. Use of a trash pump or dewatering pump capable of pumping very muddy water or suspensions of water and mud is recommended. The mixture of water and soil or sediment should be recirculated for about six to eight hours. The action of the pump impeller will help to break up soil particles to smaller sizes that do not settle rapidly.

After the turbidity slurry has been mixed as described above and then settled for one hour to allow small gravel, sand, and grit to settle to the bottom of the batch tank, the slurry can be transferred to a very large tank having the capacity in the range of 10,000 to 15,000 gallons. The diluted suspension should be stirred or recirculated using a gasoline-powered portable pump of the kind used for dewatering at project construction sites, or an electric powered pump of equivalent flow capacity. The objective is to mix the water and slurry with a turnover time of about one hour. This mixing should be done for about six to eight hours, followed by two hours of quiescent settling for removal of the larger particles that would settle of their own accord during treatment. After settling, the turbidity suspension can be blended into feed water to make a more turbid feed water, or depending on the size of the treatment equipment being evaluated, and the length of the filter run, the turbidity suspension in the large tank might be used directly as feed water. If the turbidity suspension was to be used directly, more uniform turbidity could be attained by transferring the suspension to a second large tank that could be continuously stirred.

Depending on the number and duration of filter runs for which highly turbid water will be needed, sequential use of two large tanks may be appropriate. In such a situation, one large tank would be used for stirring and settling the turbidity slurry, while the second large tank would be used as the source of turbid water for spiking or as the source of feed water.

As an alternative to the use of the 10,000 to 15,000 gallon tanks described above, a second tank in the size range of 400 to 1000 gallons could be used. In this case, the suspension that had been mixed in the first 400 to 1000 gallon tank would be settled for two hours in the original tank, and about 80% of the contents would be decanted from the first tank to the second tank, leaving the sediments on the bottom undisturbed. The second tank should be stirred to maintain the turbidity-causing particles in suspension. The suspension that has been transferred to the second tank could be fed as a concentrated suspension and thoroughly mixed into the source water to create the turbid feed water. In this approach to turbidity spiking, an in-line mixer should be used to ensure effective mixing of the turbidity suspension and the source water. Sampling of feed water for turbidity analysis should be done only after the spiked turbidity suspension is thoroughly mixed into the feed water. After the turbidity suspension has been transferred to the second tank where the suspension can be used for spiking, preparation of another batch of turbidity suspension could begin again in the first tank.

The size of the tanks and the amount of soil or sediment slurry originally prepared in the highly concentrated form in the first mixing tank (the 400 to 1000 gallon tank described above) may be influenced by the rate of flow of the treatment equipment being tested, and by the level of turbidity the FTO is trying to attain. Use of treatment equipment with larger flows, and selection of high turbidity goals may result in the need for bigger tanks and pumps and the use of considerably more soil, silt, or sediment. An estimate of the amount of soil could be made by estimating the mass concentration of suspended solids needed to produce a desired turbidity. In making such an estimate, though, the FTO should consider that a substantial portion of the soil might not be broken up into particles so fine that they do not settle out in the recommended settling times. Therefore, soil usage estimates based on suspended solids would understate actual soil requirements.

The turbid water fed in the treatment testing could be characterized by particle counting, in addition to turbidity measurement. In many cases this would require dilution of the turbid samples. A simpler test would be to collect a sample of the water and place it in a 1000 mL graduated cylinder, and then record the location of the interface between turbid water and clearer water over a period of three to five hours as the suspension settles. A turbidity suspension that settled very slowly would be representative of turbid water containing fine particulate matter that would be found in many surface waters after heavy runoff.

11.4.5 Removal of Simulated Distribution System DBP Precursors (Optional Task)

During the steady-state operation of the testing period, optional simulated distribution system (SDS) DBP testing will be performed on the membrane feed water and the filtrate product water to determine the precursor removal capabilities of the membrane system. SDS DBP testing will be used to estimate by-product formation (primarily trihalomethanes and haloacetic acids). This SDS method shall be performed by spiking a water sample with a disinfectant and holding the sample in the dark at the uniform formation conditions (UFC) specified in the Information Collection Rule (ICR) Manual for Bench- and Pilot-Scale

Treatment Studies. Alternatively, the conditions selected for SDS evaluation may be those that most closely approximate the detention time and chlorine residual found in the distribution system at the location of verification testing. (Refer to the SDS test protocol in the QA/QC section of this TSTP for further details.) The following UFC will be used for DBP formation testing:

- Incubation period of 24 +/- 1 hour;
- Incubation temperature of 20 +/- 1.0°C;
- Buffered pH of 8.0 +/- 0.2; and
- 24-hour chlorine residual of 1.0 +/- 0.4 mg Cl₂/L.

11.5 Evaluation Criteria and Minimum Reporting Requirements

- Turbidity, particle concentrations and particle removal:
 - Plot graph of feed and filtrate turbidity at four hour intervals over time during each 30 day period of operation;
 - Plot graph of feed and filtrate particle concentrations at four hour intervals over time during each 30 day period of operation;
 - Plot graph of log removal of particles between feed water and filtrate water at one-day intervals over time during each 30 day period of operation; and
 - Perform mass balance calculations of TSS through the membrane system and calculate concentrations of TSS in the backwash wastewater. Calculated values shall be compared with actual measured TSS concentrations in backwash waste. (These backwash TSS concentrations may be an important consideration for residuals disposal.)
- Water quality and removal goals specified in the PSTP:
 - Provide feed and filtrate levels for TOC and UV₂₅₄ absorbance in tabular form for each 30 day period of operation, and
 - Provide feed and filtrate concentrations of any measured water quality parameters in tabular form for each 30 day period of operation.
- Removal of indigenous bacteria (TC and HPC):
 - Provide feed and filtrate levels for TC and HPC bacteria in tabular form for each 30 day period of operation, and
 - Provide values for TC and HPC log removal in tabular form for each 30 day period of operation.
- Removal of DBPs (optional):
 - Provide feed and filtrate concentrations of Total Trihalomethanes (THM) and haloacetic acids (HAA6) formed during SDS testing for each 30 day period of operation.

12.0 TASK 4: REPORTING OF MEMBRANE PORE SIZE (OPTIONAL)

12.1 Introduction

One mechanism by which low pressure membranes can remove microorganisms from water is physical sieving. Those organisms that are larger than the largest “pore size” of the membrane are retained by the membrane; those that are smaller than the pore size pass through the membrane into the filtrate. Quantification of the membrane pore size distribution is one critical factor in assessing

whether a membrane has the potential to remove a microorganism from a feed water. Membrane pore size records will be provided for informational purposes only and will not be verified by the ETV Program. While it is best to characterize membranes microbially, it is still useful to compare the manufacturer's membrane pore size distribution with the measured membrane microbial removal from Task 8D (Section 16.2.8), if bench-scale microbial tests are conducted. Low-pressure membrane manufacturers report a "nominal" pore size, a size above which a specified percentage of particles of a certain nature are rejected under select conditions.

12.2 Experimental Objectives

The objective of this task is to report the 90% and maximum pore size for the membrane tested. This is a suggested task.

12.3 Work Plan

Membrane manufacturers will have determined the pore size distribution for their membranes. The 90% and maximum pore size should be reported and the general methods used for determining the values should be discussed. For some membranes, reporting nominal molecular weight cutoff may be more appropriate than pore size distribution. In these cases, the former may be reported with a description of its methods for determination.

13.0 TASK 5: MEMBRANE INTEGRITY TESTING

13.1 Introduction

Monitoring of membrane integrity is necessary to ensure that an adequate barrier is continuously being provided by the membrane surface. In this task, existing methods of direct and indirect membrane integrity monitoring are identified and explained. These described techniques may include, but are not limited to:

13.1.1 Direct Monitoring Methods

- Air pressure-hold testing;
- Diffusive air flow testing;
- Bubble point testing;
- Sonic wave sensing, and
- Water displacement test.

13.1.2 Indirect Monitoring Methods

- Particle counting, and
- Particle monitoring.

13.2 Experimental Objectives

The objective of this task is to demonstrate the methodology to be employed for monitoring membrane integrity and to verify integrity of membrane modules. Demonstration of the efficacy of either direct or indirect monitoring techniques is a requirement of this task.

13.3 Work Plan

The FTO shall clearly describe the most appropriate methods for monitoring of membrane integrity in the PSTP. The techniques listed above are intended to serve as examples of both direct and indirect methods for monitoring membrane integrity. These direct and indirect monitoring methods should be used together to provide consistent and sensitive evaluation of membrane system integrity.

13.3.1 Direct Monitoring Methods

Air Pressure-hold Test: The air pressure-hold test is one of the direct methods for evaluation of membrane integrity. This test can be conducted on several membrane modules simultaneously; thus, it can test the integrity of a full rack of membrane modules used for full-scale systems. Minimal loss of the held pressure (generally less than 1 psi every five minutes) at the filtrate side indicates a passed test, while a significant decrease of the held pressure indicates a failed test.

Diffusive Air Flow Test: The diffusive air flow test uses the same concept of the air pressure-hold test, but is performed by monitoring the displaced liquid volume due to the leaking air from compromised fiber(s). This test is more sensitive than the air pressure test because it is technically easier and is more accurate for measurement of small variations in liquid volume rather than small variations in air pressure.

Bubble Point Test: Bubble point testing can identify the fiber or seal location that is compromised in a membrane module. The test is typically performed after the compromised module is identified by a sonic sensor or any other monitoring method. After identifying the compromised fiber, it can then be isolated from the module by adding an epoxy glue to its inlet, or by inserting a pin with the same fiber diameter at the fiber inlet and outlet edges.

Sonic Sensing: Sonic sensors may also be used to detect the integrity of the membrane modules. The equipment consists of a sound wave sensor attached to a headphone. The headphones are manually placed at the top, middle, and bottom of the membrane module during the air-pressure hold test to detect any sound waves created by potential air bubbles leaking through a damaged fiber. The difference in audio sound between an intact and a compromised membrane may be identified by the equipment operators. Sonic sensing is only a qualitative tool for detecting loss of membrane fiber integrity, and therefore this test must be followed by a more quantitative method for evaluation of membrane integrity.

Water Displacement Test: The water displacement test is similar to the diffusive air flow test with the exception that the volume of water displaced as a result of an integrity breach is measured instead of the flow of air through a breach.

13.3.2 Indirect Monitoring Methods

Indirect methods of monitoring membrane integrity are those that do not evaluate the membrane itself, but rather use a surrogate parameter (such as particles) for assessing the membrane's condition. Continuous monitoring of particles in the filtrate stream is an indirect method for evaluating treatment reliability.

Several particle detection devices may be used for monitoring quality of the filtrate stream in terms of particles in the filtrate stream including: on-line and batch particle counters, and on-line particle monitors.

Particle Counting: Refer to Task 7, QA/QC for particle counting methodology.

Particle Monitoring: Particle monitoring is based on dynamic light obscuration. The instrument measures fluctuations in intensity of a narrow light beam which is transmitted through the sample. A fluctuating AC signal from a constant DC signal is measured by a detector and amplified. The monitor does not count particle sizes, but rather provides an index (ranging from 0 to 9,999) of the water quality. No calibration is required for this instrument since the output is a relative measurement of water quality. The potential advantages of this monitor are its low cost and ease of operation compared to particle counters.

13.4 Evaluation Criteria and Minimum Reporting Requirements

- Criteria established by the manufacturer and its designated FTO in selection of the integrity testing method:
 - Plot table of membrane integrity results as appropriate, and
 - Plot graph of integrity test results over time where appropriate for selected methodology.

14.0 TASK 6: DATA HANDLING PROTOCOL

14.1 Introduction

The data management system used in the verification test shall involve the use of computer spreadsheets and manual recording of operational parameters for the membrane equipment on a daily basis.

14.2 Experimental Objectives

The objective of this task is to establish a viable structure for the recording and transmission of field testing data to ensure that the FTO provides sufficient and reliable operational data to NSF for verification purposes.

14.3 Work Plan

The following procedure has been developed for data handling and data verification to be used by the FTO. Where possible, a Supervisory Control and Data Acquisition (SCADA) system should be used

for automatic entry of testing data into computer databases. Specific parcels of the computer databases for operational and water quality parameters should then be downloaded by manual importation into Excel (or similar spreadsheet software) as a comma delimited file. These specific database parcels shall be identified based upon discrete time spans and monitoring parameters. In spreadsheet form, the data shall be manipulated into a convenient framework to allow analysis of membrane equipment operation. At a minimum, backup of the computer databases to diskette should be performed on a monthly basis.

In the case when a SCADA system is not available, field testing operators shall record data and calculations by hand in laboratory notebooks. (Daily measurements shall be recorded on specially-prepared data log sheets as appropriate.) The laboratory notebook shall provide carbon copies of each page. The original notebooks shall be stored on-site; the carbon copy sheets shall be forwarded to the project engineer of the FTO at least once per week during each seasonal one-month testing period. This protocol will not only ease referencing the original data, but offer protection of the original record of results. Operating logs shall include a description of the membrane equipment (description of test runs, names of visitors, description of any problems or issues, etc.); such descriptions shall be provided in addition to experimental calculations and other items.

The database for the project shall be set up in the form of custom-designed spreadsheets. The spreadsheets shall be capable of storing and manipulating each monitored water quality and operational parameter from each task, each sampling location, and each sampling time. All data from the laboratory notebooks and data log sheets shall be entered into the appropriate spreadsheet. Data entry shall be conducted on-site by the designated field testing operators. All recorded calculations shall also be checked at this time. Following data entry, the spreadsheet shall be printed out and the print-out shall be checked against the handwritten data sheet. Any corrections shall be noted on the hard-copies and corrected on the screen, and then a corrected version of the spreadsheet shall be printed out. Each step of the verification process shall be initiated by the field testing operator or engineer performing the entry or verification step.

Each experiment (e.g. each membrane test run) shall be assigned a run number which will then be tied to the data from that experiment through each step of data entry and analysis. As samples are collected and sent to state-certified or third party- or EPA-accredited laboratories, the data shall be tracked by use of the same system of run numbers. Data from the outside laboratories shall be received and reviewed by the field testing operator. These data shall be entered into the data spreadsheets, corrected, and verified in the same manner as the field data.

15.0 TASK 7: QUALITY ASSURANCE/QUALITY CONTROL

15.1 Introduction

QA/QC for the operation of the membrane equipment and the measured water quality parameters shall be maintained during the verification test.

15.2 Experimental Objectives

The objective of this task is to maintain strict QA/QC methods and procedures during the verification test. When specific items of equipment or instruments are used, the objective is to maintain the operation of the equipment or instructions within the ranges specified by the manufacturer or by

Standard Methods. Maintenance of strict QA/QC procedures is important, in that if a question arises when analyzing or interpreting data collected for a given experiment, it will be possible to determine exact conditions at the time of testing.

15.3 Work Plan

When developing the Quality Assurance Project Plan (QAPP) within the PSTP, the FTO should refer to Chapter 1, Section 6.0 Quality Assurance Project Plan, in addition to the information provided herein. All of the requirements and guidelines described in Chapter 1 shall be included in the development of the PSTP. In addition to the general ETV Program QA/QC described in Chapter 1, the PSTP shall incorporate the specific adsorptive media QA items detailed in this section.

Equipment flowrates and associated signals should be documented and recorded on a routine basis. A routine daily walk through during testing shall be established to check that each piece of equipment or instrumentation is operating properly. Particular care shall be taken to confirm that any chemicals are being fed at the defined flowrate into a flowstream that is operating at the expected flowrate and that the chemical concentrations are correct. In-line monitoring equipment such as flowmeters, etc. shall be checked to confirm that the readout matches with the actual measurement (i.e., flowrate) and that the signal being recorded is correct. The items listed in this task are in addition to any specified checks outlined in the analytical methods.

15.4 Daily QA/QC Checks:

- Chemical feed pump flow rate checked daily volumetrically over a specific time period to confirm instrument reading;
- In-line turbidimeters flow rate checked daily volumetrically over a specific time period to confirm instrument reading;
- In-line turbidimeter readings checked daily against a properly calibrated bench-top model; and
- Batch and in-line particle counters flow rate checked daily volumetrically over a specific time period to confirm instrument reading.

15.5 QA/QC Checks Performed Every Two Weeks:

- In-line flowmeters/rotameters (check flow volumetrically over a specific period of time to confirm the instrument reading, and if necessary, clean equipment to remove any foulant buildup).

15.6 QA/QC Checks Performed Each Testing Period:

- In-line turbidimeters (clean out reservoirs, if necessary, and recalibrate);
- Differential pressure transmitters (check gauge readings and electrical signal using a pressure meter);
- Tubing (check condition of all tubing and connections, replace if necessary); and
- Particle Counters (perform check of microsphere calibration in the field).

15.7 On-Site Analytical Methods

The analytical methods utilized in this study for on-site monitoring of raw water and filtered water quality are described in the section below. In-line equipment is recommended for its ease of operation and because it limits the introduction of error and the variability of analytical results generated by inconsistent sampling techniques. In-line equipment is recommended for measurement of turbidity and for particle counting for feed water and is required for measurement of turbidity and for particle counting for filtered water.

15.7.1 pH

Analyses for pH shall be performed according to *Standard Method 4500-H⁺*. A three-point calibration of the pH meter used in this study shall be performed once per day when the instrument is in use. Certified pH buffers in the expected range shall be used. The pH probe shall be stored in the appropriate solution defined in the instrument manual. Transport of carbon dioxide across the air-water interface can confound pH measurement in poorly buffered waters. If this is a problem, measurement of pH in a confined vessel is recommended to minimize the effects of carbon dioxide loss to the atmosphere.

15.7.2 Temperature

Readings for temperature shall be conducted in accordance with *Standard Method 2550*. Raw water temperatures shall be obtained at least once daily. The thermometer shall have a scale marked for every 0.1°C, as a minimum, and should be calibrated weekly against a precision thermometer certified by the National Institute of Standards and Technology (NIST). (A thermometer having a range of -1°C to +51°C, subdivided in 0.1°C increments, would be appropriate for this work.)

15.7.3 Turbidity Analysis

Turbidity analyses shall be performed according to *Standard Method 2130* or EPA Method 180.1 with either an in-line or bench-top turbidimeter. In-line turbidimeters shall be used for measurement of turbidity in the filtrate waters, and either an in-line or bench-top turbidimeter may be used for measurement of the feed water (and concentrate where applicable).

During each verification testing period, the in-line and bench-top turbidimeters shall be left on continuously. Once each turbidity measurement is complete, the unit shall be switched back to its lowest setting. All glassware used for turbidity measurements shall be cleaned and handled using lint-free tissues to prevent scratching. Sample vials shall be stored inverted to prevent deposits from forming on the bottom surface of the cell.

The FTO shall be required to document any problems experienced with the monitoring turbidity instruments, and shall also be required to document any subsequent modifications or enhancements made to monitoring instruments.

15.7.3.1 Bench-top Turbidimeters. Grab samples shall be analyzed using a bench-top turbidimeter. Readings from this instrument shall serve as reference measurements throughout the study. The bench-top turbidimeter shall be calibrated within the expected range of sample measurements at the beginning of verification testing and on a weekly basis

using primary turbidity standards of 0.1, 0.5, and 3.0 nephelometric turbidity units (NTU). Secondary turbidity standards shall be obtained and checked against the primary standards. Secondary standards shall be used on a daily basis to check calibration of the turbidimeter and to recalibrate when more than one turbidity range is used.

The method for collecting grab samples shall consist of running a slow, steady stream from the sample tap, triple-rinsing a dedicated sample beaker in this stream, allowing the sample to flow down the side of the beaker to minimize bubble entrainment, double-rinsing the sample vial with the sample, carefully pouring from the beaker down the side of the sample vial, wiping the sample vial clean, inserting the sample vial into the turbidimeter, and recording the measured turbidity. For the case of cold water samples that cause the vial to fog preventing accurate readings, the vial shall be allowed to warm up by partial submersion in a warm water bath for approximately 30 seconds.

15.7.3.2 In-line Turbidimeters. In-line turbidimeters shall be used for measurement of turbidity in the filtrate water during verification testing and must be calibrated and maintained as specified in the manufacturer's O&M manual. It will be necessary to check the in-line readings using a bench-top turbidimeter at least daily; although the mechanism of analysis is not identical between the two instruments, the readings should be comparable. If the comparison suggests inaccurate readings, then all in-line turbidimeters should be recalibrated. In addition to calibration, periodic cleaning of the lens should be conducted using lint-free paper to prevent any particle or microbiological build-up that could produce inaccurate readings. Periodic checks of the sample flow should also be performed using a volumetric measurement. Instrument bulbs should be replaced on an as-needed basis. The LED readout should also be checked to ensure that it matches the data recorded on the data acquisition system, if the latter is employed.

15.7.4 Particle Counting

In-line particle counters shall be employed for measurement of particle concentrations in filtrate waters. However, either a bench-top or an in-line particle counter may be used to measure particle concentrations in the feed water, concentrate (where applicable) and pretreated waters (where applicable). Laser light scattering or light blocking instruments are recommended for particle counting during verification testing. However, other types of counters such as Coulter counters or Elzone counters may be considered for use if they can be configured to provide continuous, in-line monitoring for the filtrate product water stream. The following discussion of operation and maintenance applies primarily for use of laser light blocking instruments.

The following particle size ranges shall be monitored by both in-line and bench-top analytical instruments during the verification testing:

- 2-3 μm ;
- 3-5 μm ;
- 5-7 μm ;
- 7-10 μm ;
- 10-15 μm ; and
- 15 μm .

The FTO shall be required to document any problems experienced with the monitoring particle counting instruments, and shall also be required to document any subsequent modifications or enhancements made to monitoring instruments.

Use of particle counting to characterize feed water and filtered water quality is required as one surrogate method for evaluation of microbiological contaminant removal.

15.7.4.1 Bench-top Particle Counters. All particle counting shall be performed on-site. The particle sensor selected must be capable of measuring particles as small as 2 μm . There should be less than a 10% coincidence error for any one measurement.

Calibration. Calibration of the particle counter is generally performed by the instrument manufacturer. The calibration data will be provided by the manufacturer for entry into the software calibration program. Once the data has been entered it should be verified using calibrated commercially-available particle standards or methods. This calibration shall be verified at the beginning of each verification testing period.

Maintenance. The need for routine cleaning of the sensor cell is typically indicated by: 1) illumination of the sensor's "cell" or "laser" lamps, 2) an increase in sampling time from measurement to measurement, or 3) an increase in particle counts from measurement to measurement. During the ETV test, the sensor's "cell" and "laser" lamps and the sampling time will be checked periodically. The number of particles in the "particle-free water" will also be monitored daily.

Particle-Free Water System. "Particle-free water" (PFW) will be used for final glassware rinsing, dilution water, and blank water. This water will consist of de-ionized (DI) water that has passed through a 0.22- μm cartridge filtration system. This water is expected to contain fewer than 10 total particles per mL, as quantified by the on-site particle counter.

Glassware Preparation. All glassware used for particle counting samples shall consist of beakers designed specifically for the instrument being used. Glassware will be cleaned after every use by a triple PFW rinse. Sample beakers will then be stored inverted.

Dedicated beakers will be used at all times for unfiltered water (raw, pre-oxidized, flocculated), diluted unfiltered water, filtered water, and PFW. When several samples are collected from various equipment sampling points during one day, the appropriate beakers will be hand-washed as described above, and then rinsed three times with sample prior to collection.

Other materials in contact with the samples, including volumetric pipettes, volumetric flasks, and other glassware used for dilution, will also be triple-rinsed with both PFW and sample between each measurement.

Sample Collection. Beakers should be rinsed with the sample at least three times prior to sample collection for particle counting. Sample taps should be opened slowly prior to sampling. Sudden changes in the velocity of flow through the sampling taps should be avoided immediately prior to sample collection to avoid scouring of particles from interior surfaces. A slow, steady flow rate from the sample tap will be established and maintained for at least one minute prior to sample collection. The sample will be collected by allowing the

sample water to flow down the side of the flask or beaker; thereby minimizing entrainment of air bubbles.

Dilution. The number of particles in the raw and pretreated waters (where applicable) is likely to exceed the coincidence limit of the sensor. If so, these samples will be diluted prior to analysis. In all cases, PFW will be used as dilution water. When necessary, dilutions will be performed as follows:

- Dilution water will be dispensed directly into a 500-mL volumetric flask;
- A volumetric pipette (i.e. 10-mL for a 50:1 dilution) will be used to collect an aliquot of the sample to be diluted (stock);
- The appropriate volume of the stock will be slowly added to the volumetric flask containing the dilution water;
- The volumetric flask will be slowly filled to the full-volume etch with dilution water; and
- The volumetric flask will be inverted gently and then its contents will be poured slowly into the appropriate 500-mL flask for analysis.

During each of the above steps, care will be taken to avoid entrainment of air bubbles; thus, samples and dilution water will flow slowly down the side of containers to which they are added. Excessive flow rates through pipette tips, which can cause particle break-up, will be avoided by use of wide-mouth pipettes. Sample water will be drawn into and out of pipettes slowly to further minimize particle break-up.

Actual particle counts in a size range for diluted samples will be calculated based on the following formula:

$$\text{Sample Particle Concentration} = \frac{\{MP - (1 - X) \times PF\}}{X}$$

where MP is the measured particle concentration in the diluted sample, PF is the measured particle concentration in the particle-free water, and X represents the dilution factor. For a 25:1 dilution, the dilution factor would be 1/25, or 0.04. The expression for the dilution factor is provided by the following equation:

$$\text{Dilution Factor} = X = \frac{\text{Volume Sample}}{\text{Addition of Volume Sample} + \text{Volume Dilution Water}}$$

Particle Counting Sample Analysis. To collect samples for particle counting, at least 200 mL of each water sample to be counted (diluted or not) should be collected in the appropriate beaker. The beaker will be placed into the pressure cell and counting will take place in the “auto” mode of the instrument. Four counts will be made of each sample. The first count will serve to rinse the instrument with the sample; data from this count are discarded. Data from the subsequent three counts will be averaged, and the average value will be reported as the count for that sample.

15.7.4.2 In-line Particle Counters. In-line particle sensors selected for use must have capabilities for measurement of particles as small as 2 μm and have a coincidence error of

less than 10%. The particle counter manufacturer shall provide data and methods that the in-line particle sensors meet these criteria or an independent third party shall check that the in-line particle sensor meets the above criteria. The particle counter manufacturer shall provide the methods for demonstration of coincidence error.

The sensors of the in-line units must also be provided with a recent (two months before the start of testing) manufacturer calibration. The calibration shall be verified by measurement of the individual and cocktail suspensions of the monospheres as described for the batch counter; however, in this case the samples must be fed in-line to the counters.

No dilution of the filtered water samples shall be conducted. The data acquired from the counters shall be electronically transferred to the data acquisition system. If it is known that a particular sensor will not be used for a period of several days or more, refer to the manufacturer recommendations for an appropriate storage protocol.

15.8 Organic Parameters: TOC and UV₂₅₄ Absorbance

Samples for analysis of TOC and UV₂₅₄ absorbance shall be collected in glass bottles supplied by the laboratory and shipped at 4°C to the analytical laboratory. These samples shall be preserved, held, and shipped in accordance with *Standard Method 5010B*. Storage time before analysis shall be minimized, according to *Standard Methods*.

15.9 Microbial Parameters: TC and HPC Bacteria

Samples for analysis of TC and HPC bacteria shall be collected in bottles supplied by the laboratory and shipped with an internal cooler temperature of approximately 4°C to the analytical laboratory. Samples shall be processed for analysis by the state-certified or third party- or EPA-accredited laboratory within the time specified for the relevant method. Laboratory shall keep the samples at approximately 4°C until initiation of analysis. TC densities shall be reported as most probable number per 100 mL (MPN/100 mL) or as TC densities per 100 mL. HPC densities shall be reported as colony forming units per milliliter (cfu/mL).

15.10 Inorganic Samples

Inorganic chemical samples, including, alkalinity and hardness, shall be collected and preserved in accordance with *Standard Method 3010B*, paying particular attention to the sources of contamination as outlined in *Standard Method 3010C*. The samples shall be refrigerated at approximately 4°C immediately upon collection, shipped in a cooler, and maintained at a temperature of approximately 4°C during shipment. Samples shall be processed for analysis by a laboratory within 24 hours of collection. The laboratory shall keep the samples at approximately 4°C until initiation of analysis.

15.11 SDS DBP Test Protocol

The SDS DBP test simulates full-scale disinfection by spiking a water sample with a disinfectant and holding the spiked sample in the dark at a designated temperature and contact time. For this testing, one of two SDS approaches may be employed. The conditions selected for SDS evaluation may be those that most closely approximate the detention time and chlorine residual found in the distribution system at the location of verification testing. Alternatively, the UFC specified by the ICR may be

adopted. The UFC, as specified under the ICR stipulate that the following set of conditions will be employed:

- Incubation period of 24 +/- 1 hour;
- Incubation temperature of 20 +/- 1.0 °C;
- Buffered pH of 8.0 +/- 0.2; and
- 24-hour chlorine residual of 1.0 +/- 0.4 mg Cl₂/L.

For each SDS sample, three incubation bottles will be set up. At the end of the incubation period, each sample will be analyzed for the final disinfectant residual and the sample with the residual closest to the 1.0 +/- 0.4 mg/L range will be used for specified DBP analyses.

One liter, amber colored bottles with Teflon lined caps will be used to store the SDS samples during incubation. These bottles will be stored in a temperature-controlled incubator at the specified temperature.

All glassware used for preparation of the reagents will be chlorine demand free. Chlorine demand free glassware will be prepared by soaking glassware in a 50 mg/L chlorine bath for a period of 24 hours. At the end of this time, all glassware will be rinsed three times with organic-free water that has a TOC concentration of less than 0.2 mg/L. Glassware will then be dried at room temperature for a period of 24 hours. During the drying process, bottle openings will be covered with aluminum foil to prevent contamination.

Reagents will be prepared as follows:

15.11.1 Chlorine Stock Solution Preparation

The stock solution is prepared by adding an estimated volume of 6% reagent-grade NaOCl into a 500-mL, chlorine demand free, bottle containing an estimated amount of organic-free water. To minimize the dilution error, the chlorine stock solution is required to be at least 50 times stronger than the chlorine dose required.

15.11.2 Preparation of Additional Chemicals

Refer to *Standard Method* 4500-Cl F for the preparation method of DPD indicator, FAS standard and buffer solution. The phosphate buffer solution should be prepared as instructed in *Standard Method* 4500-Cl F.

15.11.3 Sample Collection and Incubation

The samples will be collected in a 1-L amber bottle and stored in the dark at the predetermined temperature. Samples will be adjusted to the designated pH and chlorine residual for the distribution system at the chosen site. In the case that the UFC are adopted for SDS testing, the samples will be adjusted to pH 8.0 +/- 0.2 using 1M HCl or NaOH and will then be dosed with the appropriate dosage of chlorine to yield a chlorine residual of 1.0 +/- 0.4 mg Cl₂/L after the specified 24-hour storage period. The samples will be capped head-space free and stored for the appropriate time (24 hours for UFC) in the dark at the appropriate incubation temperature.

15.11.4 Analytical Measurements

Residual free chlorine measurements will be conducted according to *Standard Methods* 4500-Cl G. DPD Colorimetric Method. Specific parameters to be measured and recorded are outlined in the specific task descriptions.

16.0 TASK 8: MICROBIAL REMOVAL

The manufacturer shall choose to have either a field microbial seeding study or bench-scale microbial testing performed as part of its ETV testing. Section 16.1 outlines the requirements for a field seeding study and Section 16.2 outlines the requirements for a bench-scale test.

16.1 Field Seeding Study

16.1.1 Introduction

Absolute removal of *Giardia* and *Cryptosporidium* has been well documented for only a selected number of MF and UF processes. Virus removal capabilities have not been well documented extensively for membrane processes. In this task, the effectiveness of membrane processes for microbial removal shall be evaluated by use of seeding studies. The field seeding studies, if chosen, shall be conducted with protozoa (*Giardia* and *Cryptosporidium*) and/or MS2 virus, and shall be performed during the required test runs conducted for Task 1.

16.1.2 Experimental Objectives

The experimental objective of this task is to characterize the membranes in terms of microbial removal. The type of seeding studies (protozoa, viruses or both) conducted as a part of this task will be left to the discretion of the manufacturer.

16.1.3 Work Plan

During the seeding studies, the FTO shall conduct the microbial seeding studies in the field as described in the following procedures and sample collection sections. The FTO shall then submit collected seeding water samples to a state-certified or third party- or EPA-accredited laboratory for microbial testing.

16.1.3.1 Organisms Employed for Field Challenge Experiments. Table 5 presents the different microorganisms that may be used for the field microbial rejection studies. Two protozoan cysts and one virus were identified for use in these seeding studies. These organisms were chosen to provide some variety in the types and sizes of microorganisms to indicate the range of membrane microbial removal capabilities. *Giardia* cysts were selected since this microorganism is one of the driving forces behind the SWTR. The model microorganism used may either be *Giardia muris*, a non-pathogenic species, or *Giardia lamblia*, a pathogenic species. *Cryptosporidium* is another important protozoan that is potentially targeted for regulation in the future. *Cryptosporidium parvum* is recommended for use in these studies.

Table 5. Microorganisms Recommended for Microbial Seeding

Microorganism	Model	Source
Protozoa	<i>Giardia muris</i>	seeded
	<i>Cryptosporidium parvum</i>	seeded
Virus	MS2 bacteriophage	seeded

MS2 bacterial virus was identified for use as the model virus for the microbial challenge studies. MS2 bacteriophage is the virus of choice for challenge studies because it is similar in size (0.025 μm), shape (icosahedron) and nucleic acid (RNA) to polio virus and hepatitis. This bacterial virus is the suggested organism to use in the SWTR Guidance Manual when conducting studies of microbial removal (USEPA, 1989).

It is recognized that in many cases it may not be possible to employ viable protozoan cysts and oocysts for seeding studies, depending upon where the equipment verification is being performed. In such a case, *Cryptosporidium* organisms fixed in no more than 10% formalin may be used. *Giardia* organisms fixed in no more than 5% formalin may be used. Alternatively, the organisms may be heat-fixed. Introduction of surrogates or alternatives for formalin- or heat-fixed protozoa and MS2 virus to this testing plan shall be based upon peer-reviewed studies and proven experimental methodologies and shall only be allowed after approval from NSF. Organism stocks received from appropriate suppliers shall be stored under refrigeration in the dark at 4°C until use in the seeding studies. Aliquots for use in each seeding study shall then be delivered on ice to the equipment on the day of the testing.

16.1.3.2 Microbial Seeding Protocols. Microbial challenges shall be conducted as batch seeding tests, with one seeding study conducted per testing period. In the batch testing mode, each microorganism to be used for challenge testing shall be seeded to a constant volume of feed water (potentially 50 to 200 gallons). Sufficient volume of stock suspension shall be created in the seeding tank to sustain membrane operation for a minimum of 30 minutes. For the protozoa seeding studies, the final seeding concentration in the feed water tank should be high enough to demonstrate at least 4 log removal of *Giardia* and *Cryptosporidium*. For the virus seeding studies, the final seeding concentration in the feed water tank should also be high enough to demonstrate at least 4 log removal of viruses. In order to show a 4 log removal of microorganisms, it is recommended that feed water contain 10^6 to 10^7 microorganisms in a challenge test.

The seeding experiments shall be conducted under the operating conditions in which the microorganisms would be most likely to penetrate the membrane. These conditions may include the high flux employed during the testing period. Initiation of the seeding study shall occur immediately after backwashing the membrane. Furthermore, the membrane seeding studies should be performed as soon as possible following a chemical cleaning procedure. If the membrane equipment is operated with automatic backwash routines, the addition of seed microorganisms should be performed immediately at the conclusion of a backwash routine to evaluate microbial removal in the absence of a cake layer on the membrane surface. The frequency of backwash may need to be adjusted during microbial challenge to allow sufficient time for sample collection.

The feed suspension of protozoa or viruses shall be prepared in the seeding tank by adding the concentrated stock suspension(s) of organisms into a feed water reservoir. The reservoir

shall be completely mixed during preparation of the seeded feed water and throughout the filtration period. After the addition of protozoa or viruses to the seeding tank and before the initiation of filtration, samples shall be collected to establish the initial titer of the microorganisms. Once filtration has begun, transmembrane pressure, filtrate flux and recirculation rate (where appropriate) shall be recorded. Sample volumes of the feed water, filtrate water and backwash water shall be recorded. An EPA-accredited laboratory shall be selected for analysis of appropriate microbial species, and sample volumes shall be processed according to the instruction provided by the EPA-accredited laboratory. At the end of sample preparation, the prepared samples shall be shipped to the EPA-accredited laboratory for analysis.

During the protozoa studies, a minimum of one sample from the feed water and three samples of the filtered water shall be prepared per seeding study (per season) for analysis by the EPA-accredited laboratory. During MS2 viral seeding studies, a minimum of one sample from the feed water, three samples from the filtrate water and one sample from the backwash water shall be collected. The first permeate sample for viral seeding studies shall be collected within the first 30 seconds of initiating filtration of the seeded waters, and subsequent samples shall be collected at 10 to 15 minute intervals. Each sample shall be collected in sterile 250 mL bottles. Bottles shall be stored at 4°C and processed within 24 hours.

16.1.4 Analytical Schedule

16.1.4.1 Water Quality Sampling. During microbial seeding studies, sampling of feed waters and filtrate waters shall be performed with daily measurement of temperature (feed only), pH, turbidity and particles.

16.1.4.2 Operational Data Collection. Operational data, as required by Task 1 shall be collected at the time of each seeding experiment.

16.1.5 Evaluation Criteria and Minimum Reporting Requirements

- Removal of *Giardia* and *Cryptosporidium*:
 - Provide feed water and filtrate levels of *Giardia* and *Cryptosporidium* in tabular form;
 - Create bar chart of log removal of microorganisms seeded (*Giardia* and *Cryptosporidium*).
- Removal of virus:
 - Provide influent and effluent levels of *virus* in tabular form, and
 - Create bar chart of log removal of microorganisms seeded (*viruses*).

16.2 Bench-Scale Characterization of Membranes Using Microbial Profiling

16.2.1 Introduction

One of the primary drivers for the use of low-pressure driven membranes for treatment of drinking water supplies has been the increased emphasis on the removal of microorganisms. Low-pressure membrane processes have been classified traditionally as MF or UF. There is currently no agreement on specifications that distinguish MF from UF. The traditional method for distinguishing UF from MF is pore size distribution or molecular weight cutoff.

MF membranes are often considered to have pore sizes ranging from 0.05 μm to 5 μm and UF membranes from 0.005 μm to 0.05 μm . There is considerable overlap between where one may consider MF to begin and UF to end. Moreover, pore size distribution does not provide an accurate or empirically based method to predict microbial removal. The actual classification of these membranes for product marketing relies primarily upon the manufacturer. Of particular note, microbial removal does not currently play a role in determining whether a membrane is classified as MF or UF. This point leads to confusion in the water community as to the classification of low-pressure membranes. If membranes are to be employed on a more widespread basis for microbial removal, then their classification should be based on their capability to remove microorganisms, not on their pore size distribution.

Microbial removal is usually evaluated through pilot testing. However, rigorous microbial challenge studies at pilot scale are often prohibitively costly. Since pilot studies are typically conducted at water facilities, opportunities for microbial challenge studies may be limited because of the potential hazard of working with microorganisms in proximity to drinking water supplies. Further, membranes are most vulnerable to microbial passage when they are first put online. There are numerous complexities of sampling for microbial agents immediately after pilot plant startup (such as collecting a sample rapidly and aseptically). The inability to sample a clean membrane as soon as it is placed online may provide inconsistent results due to cake layer accumulation or pore constriction from adsorption of natural organic matter onto the membrane.

This TSTP provides a protocol to evaluate microbial removal by membranes at bench scale. Materials for most low-pressure membrane filtration modules used in drinking water treatment have a hollow fiber geometry. Nonetheless, this TSTP can be employed for other geometries (i.e., tubular) with little modification. Its intent is to provide a widely accepted, standardized methodology with which to characterize membranes from a microbial perspective. The absence of natural water constituents in the feed will allow accurate assessment of the capability of membrane materials to remove microorganisms.

The TSTP is designed for scientific rigor, but also for ease of implementation by a qualified laboratory, denoted as laboratory testing organization throughout this document. The data generated from execution of the protocol are intended to provide utilities, engineers and regulators with the necessary information to make informed decisions about current and future membrane products that are being (or will be) employed for treatment of water supplies. As an option outside of ETV testing, these procedures may also be used by the manufacturer to determine the membrane lot acceptance of a membrane filtration media when challenge testing is needed to demonstrate removal for a lot acceptance.

16.2.2 General Approach

The general approach to the membrane bench testing is to conduct microbial challenge studies under conditions that the microorganisms are most likely to penetrate the membrane using a standardized Low-Pressure Membrane Testing Unit. The primary operational variable shall be transmembrane pressure, which shall be applied under direct flow conditions. Feed water containing the selected microorganisms shall be applied continuously to the membrane for the duration of the challenge. No backwashing or recirculation shall be employed during the experimental run. The challenge studies shall be executed through a

series of tasks as noted below and discussed in more detail after a discussion of the membrane testing unit experimental setup.

Task 8A – Establish Filtrate Flux;

Task 8B – Perform Membrane Cleaning and Backwashing;

Task 8C – Perform Membrane Integrity Testing;

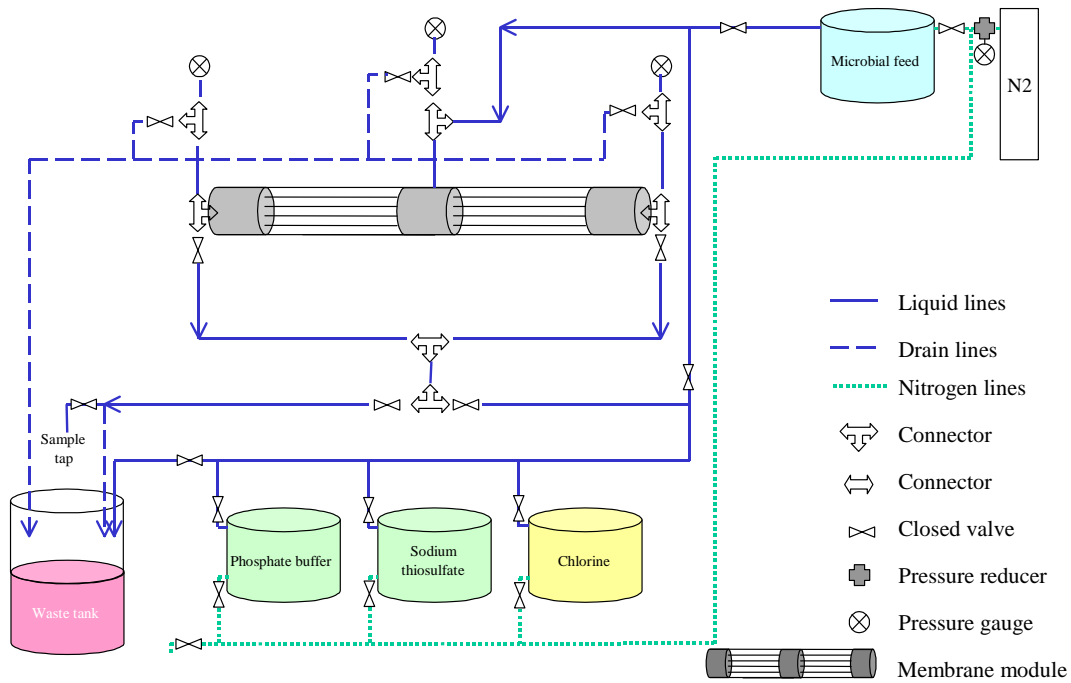
Task 8D – Conduct Microbial Removal Experiments; and

Task 8E – Execute Data Handling Protocol.

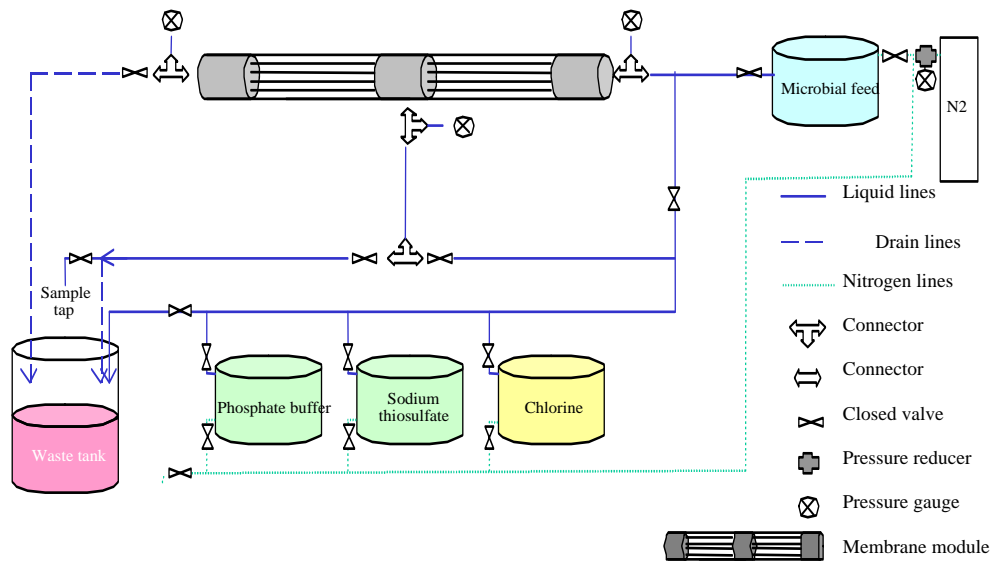
16.2.3 Membrane Filtration Unit Experimental Set Up

16.2.3.1 Low-Pressure Membrane Testing Unit. The low-pressure membrane testing unit in this document refers to the membrane module, associated tubing and connections, pressure gauges, tanks and pumps (or nitrogen tanks). The unit can be set up in three different ways, depending on the type of flow configuration necessary for the particular membrane module to be tested. These three different experimental setups for the low-pressure membrane testing unit are illustrated in Figures 2, 3, and 4. The three setups accommodate two pressure-driven flow configurations (inside out and outside in) as well as one outside-in, vacuum driven configuration. The same low-pressure membrane testing unit can be employed for each flow configuration with minor tubing changes.

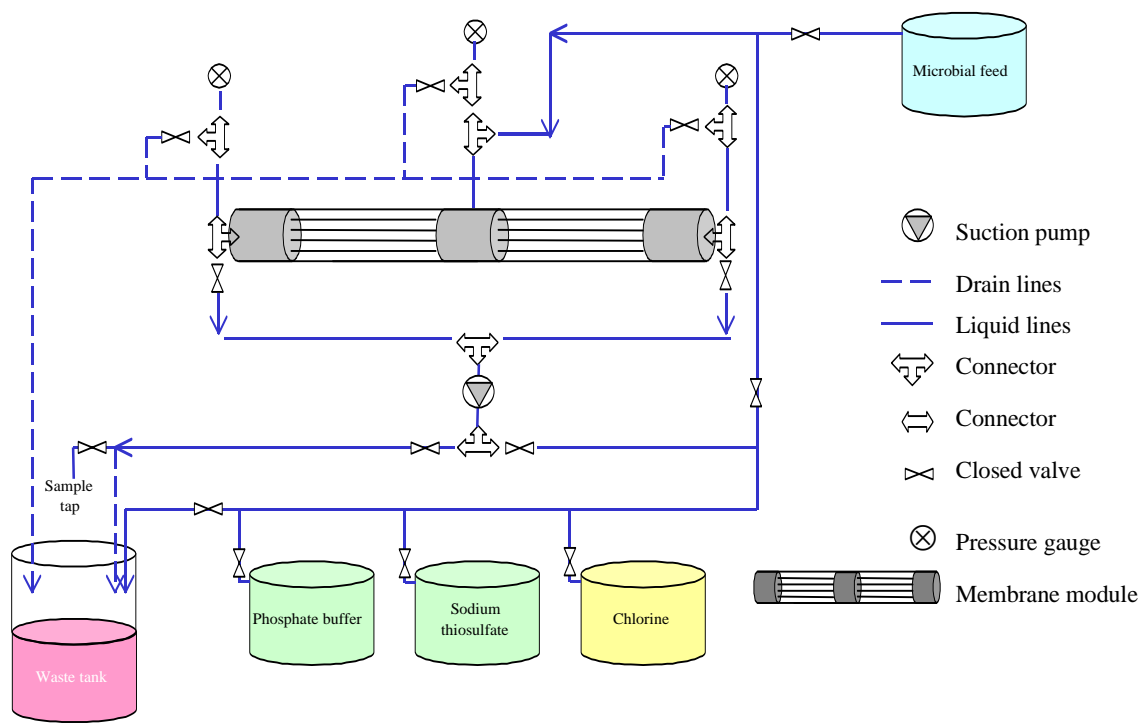
The low-pressure membrane testing unit is comprised of five separate tanks: one tank provides a microbial feed for the challenge studies; three other tanks (chlorine or other biocidal agent, sodium thiosulfate and phosphate buffer) are employed for backwashing and/or cleaning the membrane module between experimental runs. A fifth tank is used to collect waste. Pressurized nitrogen gas is employed to provide system pressure, since mechanical pumping can cause perturbations in pressure application. A vacuum system is employed for submersible vacuum driven applications. Details of materials used for construction of the low-pressure membrane testing unit are presented in Appendix 2C.



Schematic of low pressure membrane filtration unit – Outside/In flow configuration, pressure-driven
 Figure 2



Schematic of Low-Pressure Membrane Test Unit – Inside/out flow configuration, pressure driven
 Figure 3



Schematic of low pressure membrane filtration unit – Outside/in flow configuration vacuum driven
Figure 4

16.2.3.2 Bench-Scale Modules Used in Challenge Testing. Membranes exhibit product variability, and the degree of variability will depend on a number of factors specific to the product and the manufacturing process. Furthermore, product variability is manifested in different aspects of product performance and characteristics. Membranes may exhibit variability with respect to removal efficiency, pore size distribution, bubble point, productivity, and membrane area among other characteristics. All of these aspects of product variability can be important considerations for a specific application; however, variability in removal efficiency is of primary concern in the context of challenge testing.

The membrane material used by the manufacturer to fabricate the bench modules will be obtained from full-scale modules or membrane materials from their production line. The membrane modules used for evaluation in the low-pressure membrane testing units shall be based on the statistical distribution of nondestructive performance test results, as described below, or on an alternative approach provided by the manufacturer and laboratory or FTO. If an alternative approach is desired, it shall be reviewed and approved by NSF and the EPA under the ETV Program prior to implementation. Alternative approaches shall take into account product variability in terms of efficacy of microbial removal and may depend upon the specific characteristics of the membrane.

Nondestructive Membrane Test and Quality Control Release Values. A nondestructive performance test is a physical test that characterizes a fundamental property of the membrane that can be correlated to some aspect of process performance, and which does not alter or damage the membrane. In the context of challenge testing, the nondestructive performance test must be correlated to the removal efficiency of the target organism. An example of such a test is the bubble point test, the results of which can be directly related to the size of the largest pore in a membrane. Manufacturers often use such nondestructive testing as a means of quality control and assurance, and in many cases such a test is applied to every production module. The results of such extensive testing can provide a good estimate of product variability as it relates to removal efficiency.

The nondestructive performance test is used to assure the removal efficiency of production modules in the following manner. The challenge test demonstrates the removal efficiency of the specific module(s) evaluated, and these modules are characterized through application of the nondestructive performance test that is used as part of the manufacturer's routine quality control and assurance program. The results of the nondestructive performance test applied to the module(s) evaluated during the challenge test establish a control limit for the nondestructive performance test.

The nondestructive performance serves as the basis for confirming the performance of membrane modules that are not directly challenge tested. Manufacturers that have historically performed nondestructive testing for the purpose of product quality control and assurance can use this information to characterize the variability of a product line. Additionally, a manufacturer may have established a quality control release value for the nondestructive performance test that provides a minimum cutoff for an acceptable product. Based on these considerations, selection of membrane materials used in bench-scale modules for challenge testing should be based on nondestructive performance test results.

Since the challenge test is used to establish the control limit for the nondestructive performance test that production modules must meet, it would be prudent to test modules

with nondestructive performance test results that are close to the quality control release value. The rationale behind testing a worst-case module is that it allows for fewer modules to be tested while still providing a means of verifying the removal efficiency of production modules through application of the nondestructive performance test. This approach for module selection may be especially useful when complete removal of the challenge organism is anticipated for all modules of the product line.

It should be noted that many nondestructive performance tests that are suitable for evaluating the ability of a membrane to remove small organisms, such as *Cryptosporidium*, will not apply to very small ones, such as viruses. As an example, the bubble point test cannot typically be applied at a pressure high enough to achieve a resolution on the order of the size of a virus. In such cases, other manufacturing quality control procedures would be necessary to assure virus removal capabilities of production modules. These may include internal quality control testing of the membrane media or testing for acceptance of membrane modules. For more detailed information regarding nondestructive membrane tests and quality control release values, the U.S. Environmental Protection Agency Method Guidance Manual for Membrane Filtration (USEPA, 2003) should be consulted.

Bench-Scale Membrane Module Requirements. Manufacturers shall construct modules with materials as close as possible to their quality control release values, which shall be provided in the final ETV report. Appropriate information on the nondestructive performance tests for these modules or other quality control information shall be provided. A minimum of five bench modules to be employed for each bench-scale microbial characterization shall be provided by the manufacturer. Each module shall have an effective membrane area of approximately 0.1 m². The modules shall have an effective length similar to those employed at full scale to have similar pressure drops along the membrane. It is recognized that the length of the bench module may be slightly shorter than that of a full-scale module if the materials to fabricate it are taken directly from a full-scale module. Further, it is recognized that the geometry of some tubular and spiral wound membranes may preclude the use of full scale length modules or elements. In these cases, the length necessary to provide 0.1 m² modules should be employed.

Conditioning of Membrane. Before conducting testing, the membranes shall be fully wetted according to the manufacturer's specification. After wetting, each module shall be conditioned at a typical filtrate flux (as specified by the manufacturer) for a minimum of four continuous hours before any testing begins. A 0.1 mM phosphate buffer solution (pH 7.0) shall be employed as the feed water. Specific flux shall be monitored once per hour and recorded.

16.2.4 Sequence of Events for Module Testing

Table 6 below presents the general sequence of events for module testing. These events are described in more detail in each of the tasks below.

Table 6. Sequence of Events for Low-Pressure Membrane Module Testing

Event	Comments
Conditioning period for module	Run module for 4 hours
Perform first membrane integrity test	Described in Task 8C
Set filtrate flux; determine specific flux	Conduct before and after five HRTs (described in Task 8A); chemically clean only if necessary (described in Task 8B)
Perform microbial challenge test on module	Described in Task 8D
Determine specific flux	Described in Task 8A
Perform second membrane integrity test	Described in Task 8C
Repeat same sequence with other modules	

16.2.5 Task 8A: Establish Filtrate Flux

16.2.5.1 Introduction. Bench-scale membrane operation in terms of filtrate flux will be established in this task. This task shall be conducted for each of the five membrane modules being tested. This task shall also be repeated after each chemical cleaning (see Task 8B), if chemical cleaning is necessary. Each repetition of this task involves filtration of 0.1 mM phosphate buffer solution for five hydraulic residence times (HRTs) of the low-pressure membrane test unit's module and tubing at a filtrate flux under which microorganisms would be most likely to penetrate the membrane.

16.2.5.2 Experimental Objectives. The objectives of this task are to document the operational conditions under which each of the five membrane modules will be evaluated for microbial removal and then to verify those operational conditions before and after testing of each membrane module. While five modules are the minimum to be evaluated, the testing for more modules is encouraged.

16.2.5.3 Work Plan.

Specification of Filtrate Flux. For this task, the laboratory testing organization shall specify the filtrate flux to be employed during the microbial challenge studies. The microbial challenge experiments shall be conducted at operating conditions under which the microorganisms would be most likely to penetrate the membrane. These conditions shall include the highest operational flux specified by the manufacturer for their membrane using a 0.1 mM phosphate buffer solution and direct flow hydraulic conditions. Note that the pressures to obtain the highest operational flux using a phosphate buffer feed water may not be as high as those observed under field applications. This is because materials in natural water foul the membrane over time, and thus, greater pressures are required to maintain a constant flux. However, under these conditions, microbial removals are less conservative than those under the clean water conditions specified here.

It should also be noted that some hollow fiber, and most spiral wound and tubular membranes often operate in the field under crossflow conditions. Nonetheless, direct flow still represents a worse-case scenario since membrane surface concentration polarization effects do not play

a role under the conditions in which these experiments are to be conducted. Regardless of membrane geometry, the laboratory testing organization shall clearly describe how operational filtrate flux was chosen.

It is anticipated that the filtrate flux will be constant over the time of the experiment, since it is short in duration. However, if greater than 10% specific flux decline of the membrane occurs after filtering 0.1 mM phosphate buffer for five hydraulic residence times of the low-pressure membrane test unit's module and tubing, chemical cleaning shall be performed according to manufacturer specifications. Adjustments to the operational strategy shall be made (such as a decrease in filtrate flux) as necessary. Decisions on adjustments of filtrate flux shall be made by the laboratory testing organization in consultation with the manufacturer.

Microbial challenge studies at additional operational flux conditions are at the discretion of the manufacturer and the designated laboratory testing organization. However, testing of alternate additional operational conditions shall be performed only in addition to the initial flux condition specified in the work plan.

Determination of Specific Flux. On each new module, and before and after each microbial removal experiment, the hydraulic performance of the membrane module shall be evaluated by determining its specific flux. The required parameters to calculate the specific flux include:

- Filtrate flow rate;
- Effective membrane area; and
- Transmembrane pressure.

To evaluate the filtrate flow rate of the membrane, a volume of filtrate is collected over a period of one minute.

The effective membrane surface area is determined as:

$$A = \pi \times (\text{OD}) \times (l) \times (n)$$

where: A = effective membrane surface area in m² or ft²;
 l = the length of the module in m or ft;
 OD = outside diameter (OD) of the fibers (for an outside/in flow configuration) in m or ft; and
 n = number of fibers.

For an inside/out flow configuration the effective area becomes:

$$A = \pi \times (\text{ID}) \times (l) \times (n)$$

where: A = effective membrane surface area in m² or ft²;
 l = the length of the module in m or ft;
 ID = inside diameter (ID) of the fibers in m or ft; and
 n = number of fibers.

The filtrate flux is determined empirically as:

$$J = \frac{Q_p}{A}$$

where: J = filtrate flux in L/hr/m² or gal/day/ft² (gfd);
 Q_p = filtrate flow rate in L/hr or gal/day; and
 A = effective membrane surface area in m² or ft².

In a direct filtration mode, the transmembrane pressure is calculated according to:

$$P_{tm} = P_i - P_p$$

where: P_{tm} = transmembrane pressure in bar or psi;
 P_i = pressure at the inlet of the module in bar or psi; and
 P_p = filtrate pressure in bar or psi.

The water volume transfer through the membrane per unit of membrane area and driving force is the specific flux (J_s) as described in Section 6.0 of this chapter.

The filtrate flux is normalized by dividing by the transmembrane pressure or net driving force to obtain the specific flux, which is a useful measure by which different membrane operating conditions can be compared to each other.

Temperature corrections to 20°C for filtrate flux shall be made to correct for the variation of water viscosity with temperature. A specific, empirically derived equation developed by the membrane manufacturer may be used to provide temperature corrections. Alternatively, the equation by Streeter and Wiley (1985) may be employed using the effective membrane surface area as described in Section 6.0.

16.2.5.4 Evaluation Criteria and Minimum Reporting Requirements.

- Bar graph of specific flux normalized to 20°C before and after preconditioning for five hydraulic residence times and before and after challenge testing of each module.

16.2.6 Task 8B: Assess Cleaning Efficiency

16.2.6.1 Introduction. Although not anticipated, chemical cleaning of the membrane may be necessary. As such, this task describes the general procedures for conducting chemical cleaning.

16.2.6.2 Work Plan. Cleaning chemicals and cleaning routines shall be based on the recommendations of the manufacturer. The manufacturer and its designated laboratory testing organization shall specify in detail the procedure(s) for chemical cleaning of the membranes. At a minimum, the following shall be specified:

- Cleaning chemicals and concentrations;
- Hydraulic conditions of cleaning (flow, transmembrane pressure);
- Duration of each cleaning step; and
- Initial and final temperatures of chemical cleaning solution.

Each system shall be chemically cleaned using the recommended cleaning solutions and procedures specified by the manufacturer. After each chemical cleaning of the membranes, the system shall be restarted and the initial conditions of specific flux recovery and rejection capabilities shall be tested.

16.2.6.3 Evaluation Criteria and Minimum Reporting Requirements. At the conclusion of each chemical cleaning, the initial condition of transmembrane pressure, flow and temperature shall be recorded and the specific flux calculated. The efficacy of chemical cleaning shall be evaluated by the recovery of specific flux.

$$\% \text{ Recovery of Specific Flux} = 100 * (J_{s_{ac}} / J_{s_i})$$

where: $J_{s_{ac}}$ = Specific flux (L/(hr/m²/bar or gfd/psi,) after chemical cleaning at end of run,
and
 J_{s_i} = Initial specific flux (L/(hr/m²/bar or gfd/psi) at beginning of filtration run.

- Table of percent specific flux recoveries before and after each chemical cleaning shall be presented.

16.2.7 Task 8C: Perform Membrane Integrity Testing

16.2.7.1 Introduction. Monitoring of membrane integrity is necessary to ensure that an adequate barrier is continuously being provided by the membrane material during the challenge testing. Only direct membrane integrity monitoring shall be employed in the bench-scale testing. Examples of direct monitoring methods include, but are not limited to:

- Air pressure decay testing;
- Diffusive air flow testing;
- Water displacement test;
- Bubble test; and
- Sonic wave sensing.

A brief overview of these direct monitoring methods is provided below.

Air Pressure Decay Test (PDT): In this test, the membrane module is pressurized to approximately 15 psi from the feed side. Minimal loss of the held pressure (generally less than 1 psi every five minutes) at the filtrate side indicates a passed test, while a significant decrease of the held pressure indicates a failed test.

Diffusive Air Flow Test: The diffusive air flow test uses the same concept as the air pressure-decay test, but is performed by monitoring the displaced liquid volume due to the leaking air from compromised fiber(s). This test is more sensitive than the air pressure decay test because it is technically easier and more accurate to measure small variations in liquid volume rather than small variations in air pressure.

Water Displacement Test: The water displacement test is similar to the diffusive air flow test with the exception that the volume of water displaced as a result of an integrity breach is measured instead of the flow of air through a breach.

Bubble Test: Bubble testing can identify the fiber or seal location that is compromised in a membrane module. The test is typically performed after the compromised module is identified by a sonic sensor or any other monitoring method. After identifying the compromised fiber, it can be isolated from the module by adding an epoxy glue to its inlet, or by inserting pins of the same diameter as the fiber at the fiber inlet and outlet edges. The module can then be placed back online.

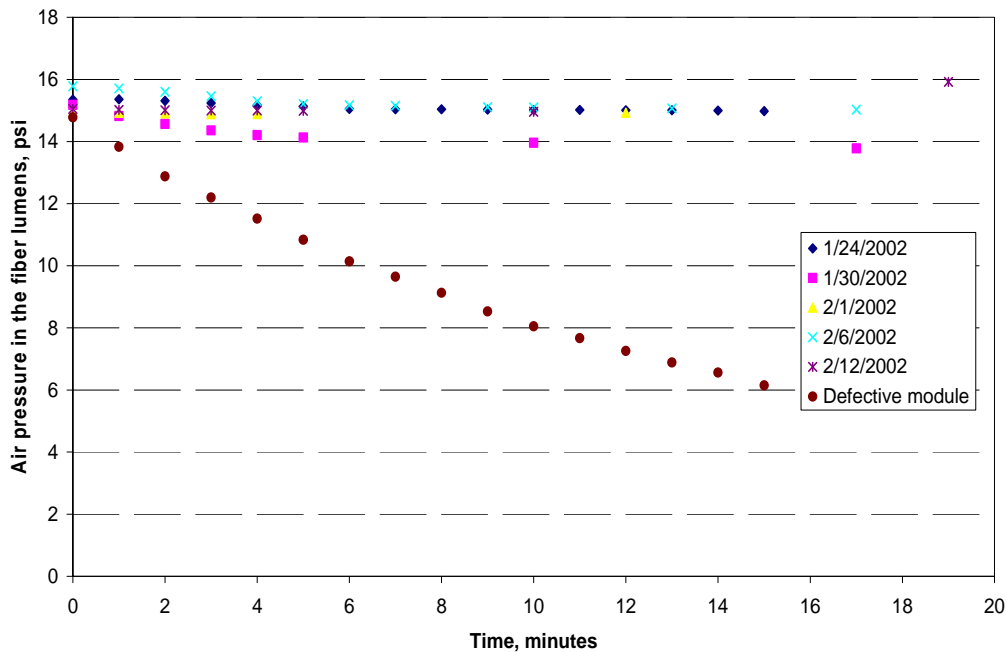
Sonic Wave Sensing: Sonic sensor equipment consists of a sound wave sensor attached to a headphone. The headphones are manually placed at the top, middle, and bottom of the membrane module during the air-pressure decay test to detect any sound waves created by air bubbles leaking through a damaged fiber. The difference in sound between an intact and a compromised membrane may be identified by the pilot operators. Sonic sensing is only a qualitative tool for detecting loss of membrane fiber integrity, and therefore this test must be followed by a more quantitative method for evaluation of membrane integrity.

16.2.7.2 Experimental Objectives. The objective of this task is to demonstrate the methodology to be employed for monitoring membrane integrity at bench scale and to verify integrity of membrane modules.

16.2.7.3 Work Plan. The laboratory testing organization shall clearly describe the most appropriate methods for monitoring of membrane integrity at bench scale. The techniques listed above are intended to serve as examples of direct methods for monitoring membrane integrity. These direct monitoring methods shall provide sensitive evaluation of membrane system integrity. It should be noted that pilot and/or full-scale methods of membrane integrity testing might have to be adapted for bench-scale applicability. Further, the bench-scale integrity monitoring does not replace integrity testing in the field. If the membrane module is shown to be compromised by integrity testing, it shall be discarded and another module shall be provided as a replacement.

Integrity testing shall be performed before and after challenge testing of each module. Since pressure decay tests (PDTs) are often used to measure membrane integrity, an example of adapting pilot integrity methodology to bench scale is described below.

PDTs are performed on each module before and after all challenge testing is completed for that module to assure the membrane module being evaluated is not compromised. To perform the test, the drain line is opened and the membrane fibers are emptied of feed solution by applying pressure from a nitrogen tank to the inner lumen of the fibers at both ends of the modules (it is important, however, to keep the membrane fibers wetted). Nitrogen air feed pressure is set at a pressure recommended by the manufacturer, after which the pressure feed line and drain line is closed. Then, the pressures at the inlet and outlet of the modules are monitored and recorded at intervals of not less than one per minute for a period recommended by the manufacturer. If the module is intact, only a small decrease in pressure is observed (usually less than 1% over a period of 15 minutes). If the membrane material or module is compromised, the PDT will show a substantial decrease of pressure over time. Figure 6 shows PDT data on both intact and compromised modules using a Low-Pressure Membrane Testing Unit.



Pressure Decay Test performed on a 10-fiber, 30-inch long membrane module

Figure 5

16.2.7.4 Evaluation Criteria and Minimum Reporting Requirements.

- Table of membrane integrity results before and after challenge testing, and
- Where appropriate for the selected integrity methodology, a temporal graph of integrity test results conducted before and after challenge testing.

16.2.8 Task 8D: Conduct Microbial Challenge Experiments

16.2.8.1 Introduction. In this task, the effectiveness of membrane materials for microbial removal shall be evaluated by use of microbial challenge studies.

16.2.8.2 Experimental Objectives. The objective of this task is to characterize the low-pressure membranes in terms of microbial removal.

16.2.8.3 Work Plan. The laboratory testing organization shall conduct the microbial seeding studies as described in the sections below.

Organisms Employed for Bench-Scale Challenge Experiments. Table 7 presents the different microorganisms that may be employed for the bench-scale microbial challenge studies. One protozoan oocyst, two bacteria and four viruses can be used for the challenge studies. These organisms were chosen to provide a wide range in types and sizes of microorganisms to create a microbial removal profile for the low-pressure membrane being challenged. The list of microorganisms in Table 7 is not a complete list and other microorganisms may be proposed for use, as circumstances require.

Table 7. Microorganisms for Microbial Challenges of Low-Pressure Membranes*

Type of Microorganism	Microorganism	Approximate size, microns
Protozoa	<i>Cryptosporidium parvum</i>	3-5
Bacteria	<i>Escherichia coli</i>	1-5
	<i>Pseudomonas diminuta</i>	0.6 –1
Virus	MS2 bacteriophage	0.027
	PRD1 bacteriophage	0.070
	hepatitis A virus	0.025
	calicivirus	0.025

* The list of microorganisms in Table 7 is not a complete list and other microorganisms may be proposed for use as circumstances require.

It is recognized that, in many cases, it may not be possible to employ viable protozoan cysts and oocysts for challenge studies, depending upon the laboratory where the work is being performed. In such a case, the organisms shall be heat-fixed. Organism stocks received from appropriate suppliers shall be stored under refrigeration in the dark at 4°C or frozen (viruses only) with appropriate preservatives until use in the challenge studies. Methods for propagation and enumeration of Table 7 organisms are described or referenced in Appendix 2B. Surrogates may only be used in the bench scale challenge tests in addition to chosen microorganisms to establish a correlation at bench scale or only when peer-reviewed studies and proven methodologies have shown the relationship between surrogates and target microorganisms.

Disinfection of Experimental System. Before performing microbial challenge experiments, the membrane module and tubing associate with the bench-scale low-pressure membrane testing unit shall be disinfected using a free chlorine solution (or other appropriate biocidal agent), which is prepared in a pressurized feed tank. The concentration of the chlorine will be as recommended by the manufacturer. The pressure in the tank shall be set at 15 psi and the membrane unit shall be operated in a backwash mode.

The membrane module and associated tubing shall be backwashed for a minimum of three hydraulic cycles with the disinfecting solution. This module and associated tubing shall then be rinsed with a 3-molar excess sodium thiosulfate solution (or other appropriate chemical) to assure any residual chlorine is quenched. The membrane module shall then be rinsed in backwash mode at 15 psi for an additional three hydraulic cycles with a 0.1 mM phosphate buffer (pH 7.0).

16.2.8.4 Microbial Challenge Experiments. The microbial challenge experiments shall be conducted under the operating conditions in which the microorganisms would be most likely to penetrate the membrane. These conditions shall include the operational flux specified by the manufacturer for their membrane using a 0.1 mM phosphate buffer solution prepared in deionized water. All challenge testing shall be conducted as batch seeding tests under direct flow hydraulic conditions. The challenge testing shall be conducted for all organisms simultaneously, i.e., all organisms shall be seeded into the feed water prior to conducting the testing. Only organisms that do not cross react should be employed. Further, tests shall be conducted to detect and quantify any microbial adsorption that occurs onto the non-membrane parts of the Low-Pressure Membrane Testing Unit. This may be accomplished by conducting a microbial challenge control test with a module that does not contain potted membranes.

For each module tested, four samples shall be collected: two discrete seeded feed tank samples (at the beginning and at end of the each test) and two discrete filtrate samples (samples may be collected sequentially, one right after the other). Thus, for the three modules evaluated, a total of 12 samples shall be collected.

Feed water to which microorganisms shall be added shall consist of a 0.1 M phosphate buffer (pH 7.0) prepared from sterile, deionized laboratory water. To check the quality of the water, measurements of pH, turbidity, particle counts, and conductivity shall be made and recorded before the seeding of any organisms. Additionally, the concentration of TOC shall be less than 0.2 mg/L. Feed water turbidity shall not exceed 0.1 NTU. Total particle counts in the 2 – 50 μm size range shall not exceed 25 per mL. Methods for these analyses are described in Appendix 2B.

The feed suspension of microorganisms shall be prepared by adding the concentrated stock suspension(s) of organisms into the feed water reservoir. For organisms that are propagated at very high titers (for MS2 and PRD1, the initial stock densities are approximately 10^{11} - 10^{12} plaque forming units/mL), one or more dilutions shall be made before adding the organisms to the feed water tank. This tank shall be completely mixed during preparation of the seeded feed water. Sufficient volume of stock suspension shall be created to sustain membrane operation for a minimum of eight hydraulic retention times per membrane module per experimental challenge test. After the addition of challenge organism(s) to the feed water tank and before the initiation of filtration, one discrete sample shall be collected from the feed water tank to establish the initial titer of the microorganisms. Each sample shall consist of collecting 35 mL of filtrate in a sterile, 50 mL polypropylene centrifuge tube (polypropylene is employed to avoid adsorption of the microorganisms onto the walls of tube). For the protozoan challenge tests, the final seeding concentration in the feed water tank shall be high enough to provide a microbial removal sensitivity limit of at least four log. For the bacterial and viral challenge tests, the final seeding concentration in the feed water tank shall also be high enough to provide a microbial removal sensitivity limit of at least 5

log. Detection limits for microorganisms used in challenge testing shall be noted in the QA/QC plan.

After collecting feed water samples, the membrane shall be operated under the hydraulic conditions (pressure and filtrate flux) established under Task 8A. The feed suspension of microorganisms shall be filtered under these conditions for a total of five hydraulic residence times to achieve steady state. At the end of this period, two discrete, consecutive samples shall be collected from the feed tank. Each sample shall consist of collecting 35 mL of filtrate in a sterile, 50 mL polypropylene centrifuge tube. At the conclusion of testing each module, a second sample shall be collected from the feed water tank. All samples shall be stored at 4°C immediately after collection. The \log_{10} mean values of the “before” and “after” feed water tank microbial densities shall be used when calculating microbial removal efficacies.

Before beginning the testing of the next previously-conditioned membrane module, the module and tubing shall be backwashed with 50 mg/L of free chlorine (or other appropriate biocidal agent) for five hydraulic cycles. The membrane shall then be rinsed with a 3-molar excess sodium thiosulfate solution (or other appropriate chemical) buffer and phosphate buffer as described above. After this procedure, the next test shall be initiated. At the end of each day, the microbial samples shall be shipped via overnight express for enumeration, if not enumerated on site.

16.2.8.5 Operational Data Collection. Operational data are collected under Task 8A.

16.2.8.6 Evaluation Criteria and Minimum Reporting Requirements.

- Table of water quality data: pH, turbidity, particle counts, and conductivity;
- Table of feed water and filtrate levels for all organisms; and
- Bar chart of log removal of microorganisms.

16.2.9 Task 8E: Execute Data Handling Protocol

16.2.9.1 Introduction. The data management system used in the bench-scale membrane characterization shall involve the use of computer spreadsheets and manual recording of operational parameters for the Low-Pressure Membrane Test Unit.

16.2.9.2 Experimental Objectives. The objective of this task is to establish a structure for the recording and transmission of laboratory testing data.

16.2.9.3 Work Plan. The following protocol has been developed for data handling and data verification by the laboratory testing organization. Specific parcels of the computer databases for operational and water quality parameters shall be entered by manual importation into Excel (or similar spreadsheet software). Backup of the computer databases to diskette, compact disk, magnetic tape or other archival format shall be performed at the end of each day.

Measurements shall be recorded on specially prepared data log sheets as appropriate. A laboratory notebook shall be used to record all data, calculations and other pertinent

information not included in the data log sheets. The laboratory notebook shall provide carbon copies of each page. The original notebooks shall be stored in the laboratory.

The database for the project shall be set up in the form of custom-designed spreadsheets. The spreadsheets shall be capable of storing and manipulating each monitored water quality and operational parameter from each task, each sampling location, and each sampling time. All data from the laboratory notebooks and data log sheets shall be entered into the appropriate spreadsheet. All recorded calculations shall also be checked at this time. Following data entry, the spreadsheet shall be printed out and the printout shall be checked against the handwritten data sheet. Any corrections shall be noted on the hard copies and corrected on the screen, and then a corrected version of the spreadsheet shall be printed out. Each step of the verification process shall be initialed by the bench testing operator, technician or engineer performing the entry or verification step.

The testing of each membrane module shall be assigned a run number that will then be tied to the data from that experiment through each step of data entry and analysis. As samples are collected, the data shall be tracked by use of the same system of run numbers. Data from the outside laboratories, if any, shall be received and reviewed by the laboratory staff conducting the studies. These data shall be entered into the data spreadsheets, corrected, and verified in the same manner as the field data.

17.0 TASK 9: RAW WATER PRETREATMENT (OPTIONAL)

17.1 Introduction

In most membrane systems employed for microbial and particle removal, there are usually no chemicals added to the raw water before filtration. However, some manufacturers may wish to be verified for a pretreatment technique that may not be considered a necessary process of the membrane technology for microbiological and particulate removal. As such, pretreatment can be employed to extend membrane operational time or remove selected contaminants. For example, some membranes are capable of absolute removal of microorganisms, but provide little or no removal of DBP precursors. Addition of a coagulant or adsorbent to the raw water may enhance the removal of these precursors.

Verification of optional or separable pretreatment techniques shall constitute an optional task in the verification testing of membrane equipment. This task shall be conducted for an additional month of testing and shall be considered a discretionary supplement to the verification test. In cases where a pretreatment technique is considered an integral or inseparable part of the function of the membrane system, no additional testing of system pretreatment capabilities would be necessary.

17.2 Experimental Objectives

The objectives of this task are to demonstrate membrane performance following a selected pretreatment technique and determine the efficacy of pretreatment for the membrane equipment tested, based upon the manufacturer's treatment goals. For the purposes of this microbiological and particulate contaminant removal TSTP, membrane operation and particulate removal shall be monitored as described in the analytical schedule below. For additional monitoring for removal of selected contaminants, however, the appropriate ETV protocols and TSTPs should be consulted. For

example, if the optional pretreatment selected is designed to achieve removal of precursors to DBPs, the ETV protocol and TSTP for removal precursors to DBPs should be consulted and the analytical schedule followed as a demonstration of equipment performance.

17.3 Work Plan

The focus of this task is to determine the relative rates of flux decline and performance capabilities of the membranes as a function of the selected pretreatment process. Appropriate pretreatment techniques shall be specified by the FTO.

17.4 Analytical Schedule

The pretreatment testing schedule shall be determined by the FTO. However, each pretreatment technique should be tested for a minimum of one month, preferably during the month immediately following the required month of testing for Tasks 1 through 3.

17.4.1 Raw, Pretreated Feed and Filtrate Water Characterization

For this TSTP addressing removal of microbiological and particulate contaminants, monitoring shall be conducted to provide a baseline of the solids removal capabilities of the pretreatment and membrane system. At the beginning of each membrane testing period at a single set of operating conditions (and thereafter with indicated frequency), the raw water, the pretreated feed water and the filtrate water shall be characterized by measurement of the following water quality parameters (as indicated in Table 3):

- Alkalinity (monthly);
- Hardness (monthly);
- TSS (once/two weeks);
- TDS (once/two weeks);
- TOC (monthly*);
- UV_{254 nm} absorbance (monthly*);
- TC and HPC bacteria (weekly);
- Temperature (daily, raw and pretreated feed only);
- pH (twice per week*);
- Filtrate water turbidity and particle concentrations (daily); and
- Raw water and pretreated feed water turbidity and particle concentrations (daily).

*Note: more frequent monitoring may be performed at the discretion of the manufacturer or FTO.

Additional monitoring may be required for characterization of the raw, pretreated feed and filtrate waters, in the case that protocols and TSTPs for other selected contaminants are employed for demonstration of pretreatment removal capabilities.

17.4.2 Water Quality Sample Collection

Water quality data shall be collected at regular intervals during each period of membrane testing, as required in Table 3. For verification of particulate removal, turbidity and particle concentrations in filtrate waters shall be monitored continuously using either batch or in-line

analytical instruments. Grab samples of raw waters and pretreated feed waters shall be measured by the FTO daily for temperature, turbidity and particle concentrations using bench-top analytical instruments. The specific particle size ranges to be monitored by both in-line and bench-top analytical instruments during the verification testing are indicated in Task 7, the QA/QC section.

TSS shall be monitored every other week and results of this analysis will be used to construct a mass balance of suspended solids through the membrane system. Monitoring of water quality characteristics such as TOC and UV_{254} absorbance shall be performed on a monthly basis to provide a general background on the source water character and quality for each testing period. Additional sampling and data collection may be performed at the discretion of the FTO. Sample collection frequency and protocol shall be defined by the FTO in the PSTP.

On a weekly basis, samples of raw water, pretreated feed water and filtrate shall be collected for analysis of indigenous bacterial densities including: TC and HPC. Collected samples shall be placed in a cooler with blue ice to be shipped with an internal cooler temperature of approximately 2-8°C to the state-certified or third party- or EPA-accredited analytical laboratory. Samples shall be processed for analysis by a laboratory that is certified, accredited or approved by a state, a third-party organization (i.e., NSF), or the EPA within 24 hours of collection. The laboratory shall then keep the samples at a temperature of approximately 2-8°C until initiation of analysis. TC densities will be reported as most probable number per 100 mL (MPN/100 mL) and HPC densities will be reported as colony forming units per milliliter (cfu/mL).

17.4.3 Feed Water Quality Limitations

The characteristics of raw waters and pretreated feed waters encountered during the one-month testing period shall be explicitly stated in reporting the membrane flux and recovery data. Accurate reporting of such feed water characteristics as temperature, turbidity, TSS, pH, alkalinity and hardness is critical for the verification testing program, as these parameters can substantially influence membrane performance on a seasonal basis.

17.5 Evaluation Criteria and Minimum Reporting Requirements

- Transmembrane pressure (P_{tm}):
 - Plot graph of transmembrane pressure over time for each 30 day period of operation.
- Rate of specific flux decline:
 - Plot graph of specific flux normalized to 20 degrees C over time for each 30 day period of operation.
- Cleaning frequency:
 - Provide table of intervals between chemical cleaning episodes during each 30 day period of operation.
- Cleaning efficacy:
 - Provide table of cleaning efficacy indicators for chemical cleaning procedures performed during each 30 day period of operation.

- Flux recovery:
 - Provide table of post cleaning flux recoveries during each 30 day period of operation.
- Turbidity, particle concentrations and particle removal:
 - Plot graph of raw water, pretreated feed, and filtrate turbidity over time during each 30 day period of operation;
 - Plot graph of raw water, pretreated feed, and filtrate particle concentrations over time during each 30 day period of operation;
 - Plot graph of log removal of particles between raw water, pretreated feed, and filtrate water at one-day intervals over time during each 30 day period of operation; and
 - Perform mass balance calculations of TSS through the membrane system and calculate concentrations of TSS in the backwash waste water. Calculated values shall be compared with actual measured TSS concentrations in backwash waste. (These backwash TSS concentrations may be an important consideration for residuals disposal.).
- Water quality and removal goals specified by the manufacturer:
 - Provide raw water, pretreated feed, and filtrate levels for TOC and UV₂₅₄ absorbance in tabular form for each 30 day period of operation, and
 - Provide raw water, pretreated feed, and filtrate concentrations of any selected water quality parameters in tabular form for each 30 day period of operation.
- Removal of indigenous bacteria (TC and HPC):
 - Provide raw water, pretreated feed, and filtrate levels for TC and HPC bacteria in tabular form for each 30 day period of operation, and
 - Provide values for TC and HPC log removal in tabular form for each 30 day period of operation.

18.0 OPERATION AND MAINTENANCE

The FTO shall obtain the manufacturer-supplied O&M manual(s) to evaluate the instructions and procedures for their applicability during the verification testing period. Below are recommendations for criteria to evaluate O&M manuals for membrane filtration equipment that are designed to achieve removal of microbiological and particulate contaminants.

18.1 Maintenance

The manufacturer shall provide readily understood information on the recommended or required maintenance schedule for each piece of operating equipment such as:

- Pumps
- Valves
- Pressure gauges
- Backwash controls;
- Flow meters;
- Air compressors;
- Chemical feeder systems;
- Mixers;

- Motors;
- Instruments, such as streaming current monitors or turbidimeters; and
- Water meters, if provided.

The manufacturer should provide readily understood information on the recommended or required maintenance for non-mechanical or non-electrical equipment such as:

- Tanks and basins;
- In-line static mixers; and
- Tubing and hoses.

18.2 Operation

The manufacturer should provide readily understood recommendations for procedures related to proper operation of the equipment. Among the operating aspects that should be discussed are:

Filtration:

- Control of feed flow to the membrane system;
- Measurement of inlet/outlet pressures and filtrate flows;
- Measurement of transmembrane pressure changes during filter run; and
- Feed flow control in response to temperature changes.

Membrane backwashing:

- Programming automated frequency;
- Proper backwash venting and disposal;
- Appropriate backwash rate (if applicable); and
- Monitoring during return of filter to service.

Chemical cleaning:

- Selection of proper chemical washing sequence;
- Proper procedures for dilution of chemicals;
- Monitoring of pH through chemical cleaning cycle;
- Rinsing of membrane system following chemical clean; and
- Return of filter to service.

Chemical feeders (in the case that chemical pretreatment is applied):

- Calibration check;
- Settings and adjustments (how they should be made); and
- Dilution of chemicals and polymers (proper procedures).

Monitoring and observing operation:

- Observation of feed water or pretreated water turbidity;
- Observation of transmembrane pressure increase between backwashes;
- Filtered water turbidity;
- Filter head loss; and
- What to do if turbidity breakthrough occurs.

The manufacturer should provide a troubleshooting guide; a simple check-list of what to do for a variety of problems including:

- No raw water (feed water) flow to plant;
- Can't control rate of flow of water through equipment;
- Valving configuration for direct flow and cross-flow operation modes;
- Poor raw water quality (raw water quality falls outside the performance range of the equipment);
- Poor filtrate quality;
- Failed membrane test;
- Low pump feed pressure;
- Automatic operation (if provided) not functioning;
- Filtered water turbidity too high;
- Head loss builds up excessively rapidly;
- Reduced filtrate flux;
- Machine will not start and "Power On" indicator off;
- Machine will not start and "Power On" indicator on;
- Pump cavitation;
- Valve stuck or won't operate; and
- No electric power.

It is also recommended that the manufacturer add a toll free number to the O&M manual for technical assistance on operation and maintenance of the equipment.

The following are recommendations regarding operability aspects of systems that are designed to achieve removal of microbiological and particulate contaminants. These aspects of plant operation should be included if possible in reviews of historical data, and should be included to the extent practical in reports of equipment testing when the testing is done under this TSTP.

During verification testing and during compilation of historical equipment operating data, attention shall be given to equipment operability aspects. Among the factors that should be considered are:

- Fluctuation of flow rates and pressures through membrane unit including the time interval at which resetting is needed (i.e., how long can feed pumps hold on a set value for the feed rate?).
- Presence of devices to aid the operator with flow control adjustment and chemical dosage selection:
 - Are influent and filtered water continuous turbidimeters provided;
 - Are continuous particle counter provided on membrane filtered water; and/or
 - Can backwash be done automatically?
- If automatic backwash provided, could it be initiated by:
 - Reaching a set value for head loss;
 - Reaching a set value for filtered water turbidity; and/or
 - A preset automatic timer?
- Does remote notification to operator occur when backwash happens?

- Can operator observe backwash?
- Does plant have multiple feed points for chemicals:
 - For pH adjustment;
 - For coagulant chemical feed; and/or
 - For antiscalant addition?
- Is transmembrane pressure measurement provided?
- Is rate of flow of raw water measured?
- Is chemical feed paced with raw water flow?
- Is backwash rate of flow measured and variable?
- Is backwash duration (time) variable?

Both the reviews of historical data and the reports on verification testing should address the above questions in the written reports. The issues of operability should be dealt with in the portion of the reports that are written in response to Tasks 1 & 2 of this TSTP addressing the removal of microbiological and particulate contaminants.

19.0 REFERENCES

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Streeter, V.L. and E.B. Wiley. 1985. *Fluid Mechanics*, 8th ed. New York, McGraw Hill Book Company.

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U.S. Environmental Protection Agency. 2003. *Draft Guidance Manual for Membrane Filtration* (To be online as a draft at <http://www.epa.gov/edocket/> in Fall 2003).

APPENDIX 2A

STATE-SPECIFIC VERIFICATION TESTING REQUIREMENTS

Ohio:

- It would be informative to determine maximum membrane pore size at the end of the testing (i.e., end of month 11) as well as at the beginning (month 1).
- Alkalinity and hardness measurement should be increased to daily.

Alaska:

- The task of reporting the membrane pore size will be required.

Missouri:

- The task of reporting the membrane pore size will be required.

APPENDIX 2B: QUALITY ASSURANCE/QUALITY CONTROL

Introduction

Quality assurance and quality control (QA/QC) of the operation of the membrane equipment and the measured water quality parameters shall be maintained during the laboratory testing program.

Operational and Low-Pressure Membrane Testing Unit QA/QC

Before the testing of each manufacturer's modules, on-line pressure gauges shall be checked with secondary gauges to confirm that the readout matches the actual measurement. Unit tubing and connections shall be inspected weekly to check that they are in good condition. Replacement of these materials shall be made as necessary.

Analytical Methods

The analytical methods utilized in this study for feed waters are described in the section below.

pH. Analyses for pH shall be performed according to *Standard Method 4500-H⁺*. A 3-point calibration of the pH meter used in this study shall be performed once per day when the instrument is in use. Certified pH buffers in the expected range shall be used. The pH probe shall be stored in the appropriate solution defined in the instrument manual.

Temperature. Readings for temperature shall be conducted in accordance with *Standard Method 2550*. The thermometer shall have a scale marked for every 0.1°C, as a minimum, and shall be calibrated biweekly against a precision thermometer certified by the National Institute of Standards and Technology (NIST). (A thermometer having a range of -1°C to +51°C, subdivided in 0.1°C increments, would be appropriate for this work.)

Turbidity Analysis. Turbidity analyses shall be performed according to *Standard Method 2130* or EPA Method 180.1 using a bench-top turbidimeter. All glassware used for turbidity measurements shall be cleaned and handled using lint-free tissues to prevent scratching. Sample vials shall be stored inverted to prevent deposits from forming on the bottom surface of the cell. Grab samples shall be analyzed using a bench-top turbidimeter. Information on calibration, verification of calibration, sampling and analysis can be found in the ETV Protocol for Equipment Testing for Physical Removal of Microbiological and Particulate Contaminants (NSF/USEPA, 2002).

Particle Counting. Bench-top particle counters shall be used to measure particle concentrations in the feed water. Laser light scattering or light blocking instruments are recommended for particle counting; however, other types of counters such as Coulter counters or Elzone counters may be considered.

The following particle size ranges shall be monitored by bench-top analytical instruments during the membrane characterization testing:

- 2-3 µm;
- 3-5 µm;
- 5-7 µm;

- 7-10 μm ;
- 10-15 μm ; and
- >15 μm .

Information on calibration, verification of calibration, maintenance of the particle counters, particle free water, sampling and analysis can be found in the *ETV Protocol for Equipment Testing for Physical Removal of Microbiological and Particulate Contaminants* (NSF/USEPA, 2002).

Conductivity. This parameter shall be measured according to *Standard Method 2510B* (1998).

Total Organic Carbon (TOC) /Dissolved Organic Carbon (DOC). TOC/DOC shall be analyzed according to *Standard Method 5310B* or *5310C* (1998).

Chlorine Preparation for Membrane Cleaning. The stock solution shall be prepared by adding an estimated volume of 6% reagent-grade NaOCl into a 500-mL, chlorine demand-free bottle containing an estimated amount of organic-free water. The concentration of the chlorine solution will be as recommended by the manufacturer. Refer to *Standard Method 4500-Cl F* for the preparation method of DPD indicator, FAS standard and buffer solution. Residual free chlorine measurements will be conducted according to *Standard Methods 4500-Cl G. DPD Colorimetric Method*.

Bacteriophages. Bacteriophages MS2 and PRD1 shall be enumerated according to National Water Research Institute and American Water Works Association Research Foundation, 2000. Because of the importance of this organism in characterizing the membrane at bench scale, detailed methods for MS2 are provided below and shall be followed.

MS2 bacteriophage Soft Agar Overlay Method and MS2 Stock Preparation. The bacteriophage MS2 – ATCC 15597-B1 shall be employed in all studies. The *Escherichia coli* C-3000 – ATCC 15597 shall be employed as the host bacterium, with the bacterial growth media being tryptic soy broth (TSB) – DIFCO 0370-15-5, or the equivalent.

Tryptic Soy Agar (bottom agar petri plates 100 x 15mm) Preparation. The media is rehydrated according to label directions. A magnetic stir bar is placed into the dehydration flask, and the media is brought to a near boil to dissolve the agar. The flask and contents are then sterilized by autoclaving for 15 minutes after which it is cooled in a water bath to between 45-50°C. Plates are poured using approximately 12–15 mL per plate. Enough agar is added to cover about 2/3 of the area of the plate. After pouring one plate, the lid is replaced on the dish and gently swirled so that the agar covers the entire bottom of the plate. Plates are allowed to remain motionless until the agar hardens (usually 10-15 minutes). Plates are stored at 4°C up to 30 days.

Tryptic Soy Agar Overlay Tubes. The media is rehydrated according to label directions. A magnetic stir bar is placed into the rehydration flask, and the media is brought to a near boil to dissolve the agar. The media is sterilized for 15 minutes and then pipeted aseptically into 15 mL tubes (3mL per tube) or pipeted into the tubes (3 mL per tube), which are then capped loosely and sterilized for 15 minutes. The caps are tightened after cooling. Overlay tubes are stored at 4°C for 30 days.

Preparation of High-Titer MS2 Bacteriophage. To propagate the MS2 bacteriophage, a bacterial host slant of *E. coli* (ATCC #15597) is washed with 3 mL of sterile TSB. The total 3 mL is then transferred to a 1-liter flask containing 200 mL of sterile TSB and incubated at 37°C for approximately 3 hours. At this time, the flask is removed from the incubator and 2 mL of

bacteriophage stock (ATCC #15597-B1) is added and then the flask is placed back in the incubator for an additional four hours. Then, 0.02 g of lysozyme and 6 mL of 0.2 M sterile EDTA are added to the flask which is shaken for an additional 30 minutes. The bacteriophage/bacteria suspension is poured into 4-50mL centrifuge tubes and centrifuged at 4,000 times gravity for 15 minutes.

During preparation of high titer MS2 bacteriophage stocks, there is a potential for aggregate formation. To reduce aggregates, the MS2 stock is filtered through sequentially smaller (0.45 micron, 0.22 micron and 0.1 micron), low protein-binding filters. To reduce MS2 binding to the filter, each filter is pretreated by filtering 10 mL of 0.1% Tween 80 followed by 10 mL reagent grade non-chlorinated water. MS2 stock preparations are filtered through these pretreated filters with careful attention focused on amount of pressure/vacuum applied to prevent membrane filter failure. Multiple filters may be necessary to filter the entire MS2 stock solution.

The bacteriophage stock is then titered to determine its concentration and stored at 4°C for up to four weeks.

Preparation of Host Culture. The host culture is started the day before the assay is to be performed. Using a sterile swab, a small amount of *E. coli* host (ATCC 15597) is removed from an agar slant and placed into a sterile tube containing 3 mL of tryptic soy broth and grown overnight at 37°C for 24 hours. The next day, 1 mL of the overnight culture is pipeted into 50 mL of tryptic soy broth in a 250 mL Erlenmeyer flask or the equivalent. The culture is then placed in a 37°C incubator for four hours, after which it is removed from incubator and place on ice until used.

Soft Agar Overlay Method for Bacteriophage. Bacteriophage in bench-scale low-pressure membrane samples are enumerated by the addition of the sample to soft or overlay agar along with a liquid culture of bacteria (host) in the log phase of growth. Overlay tubes are melted in a boiling water bath or autoclaved for five minutes and place in a 49°C water bath until used. The bottom of the petri plates are labeled with the identification of sample to be analyzed. Then 0.1 – 1 mL of the 4-hour host culture (which is in log phase of growth) is pipeted into a prewarmed overlay tube along with 0.1 –1 mL of the sample to be analyzed. The tube is mixed by rapidly rolling between the analyst's palms and poured onto a TSA plate. The sample is spread evenly over the surface of the plate by gently and quickly swirling the plate. The plate, which solidifies within 30 seconds, is then inverted and incubated at 37°C for 24 hours +/- 2 hours. The sample is then incubated for 24 hours. During the incubation time, the host bacteria forms a confluent lawn over the surface of the petri plate. The petri plate is incubated at 37°C for 24 hours. During the incubation period, the phage particles that are present in the sample attach to and infiltrate the bacterial host cells. The bacteriophages replicate within the bacterial cells and reach a concentration that lyse (burst) the bacterium. The destruction of the bacterial cells that make up the confluent lawn result in clear areas known as plaques. The concentration of bacteriophage originally present in the sample are determined by visually counting the clear areas, which are reported number of plaque forming units per mL (PFU/mL).

Giardia sp. and Cryptosporidium sp. These organisms shall be enumerated according to U.S. Environmental Protection Agency Method 1623 (1999).

Pseudomonas diminuta. This organism shall be enumerated according to ASTM Method F838-83 (1993).

Escherichia coli. This organism shall be enumerated according APHA, AWWA, and WEF (1999).

**APPENDIX 2C:
MATERIALS EMPLOYED FOR FABRICATION OF BENCH SCALE LOW-PRESSURE
MEMBRANE TEST UNIT**

(Note: if needed, contact NSF for potential source of materials.)

- Pressure vessel with vacuum closure (2-gallon volumes);
- Glass-filled nylon instant tube (“plug and play”) fitting, male pipe adapters ¼”, ¼”;
- Glass-filled nylon instant tube (“plug and play”) fitting, male 90 elbow pipe adapters ¼”, ¼”;
- Glass-filled nylon instant tube (“plug and play”) fitting, male branch tee pipe adapters ¼”, ¼”, ¼”;
- Glass-filled nylon instant tube (“plug and play”) fitting, male run tee pipe adapters ¼”, ¼”, ¼”;
- Glass-filled nylon instant tube (“plug and play”) fitting, coupling ¼”, ref 5779k14;
- Glass-filled nylon instant tube (“plug and play”) fitting, 90 elbow ¼”, ref 5779k24;
- Glass-filled nylon instant tube (“plug and play”) fitting, tee ¼”, ref 5779k34;
- Cement: All-purpose cement for PVC, ABS, CPVC and reference 30821;
- Nylon tubing: named “Nylon 6 tubing” and order in ¼” OD, ref 5173K9;
- PVC threaded pipe fitting – schedule 80 – dark gray, reducing hex bushing, NPT male ¾” x NPT female ½”, ref 4596k414;
- Miniature chrome-plated brass ball valves, female ¼”, female ¼”, wedge handle, ref 4912k47; and
- Teflon thread sealant tape, ½” width, ref 4591k12.