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Environmental Technology Verification Protocol

Drinking Water Systems Center

GENERIC PROTOCOL FOR THE PRODUCT SPECIFIC CHALLENGE TESTING OF BAG AND CARTRIDGE FILTER SYSTEMS

Prepared by

NSF International

Under a Cooperative Agreement with

U.S. Environmental Protection Agency

ETV ETV ETV

Contents

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Abbreviations and Acronyms

ATCC	American Type Culture Collection
°C	Degrees Celsius
CFU	Colony Forming Units
cm	Centimeter
DWS	Drinking Water Systems
EPA	U. S. Environmental Protection Agency
ETV	Environmental Technology Verification
°F	Degrees Fahrenheit
L	Liter
LIMS	Laboratory Information Management System
MFGM	Membrane Filtration Guidance Manual
mg	Milligram
mL	Milliliter
NIST	National Institute of Standards and Technology
nm	Nanometer
NSF	NSF International (formerly known as National Sanitation Foundation)
NTU	Nephelometric Turbidity Unit
QA	Quality Assurance
QC	Quality Control
QAPP	Quality Assurance Project Plan
QMP	Quality Management Plan
RPD	Relative Percent Deviation
TDS	Total Dissolved Solids
TGM	Toolbox Guidance Manual
TSA	Tryptic Soy Agar

Glossary

If a term is not described herein the reader should consult the EPA Membrane Filtration Guidance Manual for a complete list.

Bag and Cartridge Filters – pressure driven separation devices that remove particles larger than 1 micrometer (µm) using an engineered porous filtration media.

Challenge Particulate – the target organism or acceptable surrogate used to determine the log removal value (LRV) during a challenge test.

Filtrate – the water produced from a filtration process; typically used to describe the water produced by porous membranes such those used in membrane cartridge filtration (MCF), microfiltration (MF), and ultrafiltration (UF) process, although used in the context of the LT2ESWTR to describe the water produced from all membrane filtration processes, including nanofiltration (NF) and reverse osmosis (RO).

Flux – the throughput of a pressure-driven membrane filtration system expressed as flow per unit of membrane area (e.g., gallons per square foot per day (gfd) or liters per hour per square meter (Lmh)).

Log Removal Value (LRV) – filtration removal efficiency for a target organism, particulate, or surrogate expressed as $\log_{10}$ (i.e., $\log_{10}$(feed concentration) – $\log_{10}$(filtrate concentration)).

Terminal Pressure Drop – the pressure drop across a bag or cartridge filter at which the manufacturer states the filter should be replaced. Establishes the end of the useful life of the filter.

PREFACE and SCOPE

This document is a generic verification protocol (PROTOCOL) for challenge testing of full scale bag and cartridge filters as developed under the EPA's Environmental Technology Verification (ETV) Drinking Water Systems (DWS) Center. The purpose of this document is to describe the performance evaluation test procedure and quality assurance/quality control (QA/QC) procedures for the product specific challenge testing of full scale bag and cartridge filters for *Cryptosporidium* removal credits.

This PROTOCOL addresses bag and cartridge filter systems that meet the definition of such products in the LT2ESWTR. Bag and cartridge filters are defined as pressure driven separation processes that remove particulate matter larger than 1 micrometer using an engineered porous filtration media through either surface or depth filtration. The filters are housed in pressure vessels, and a system may consist of either single or multiple filters, arranged in series or in parallel. Filter systems shall be tested in the configuration in which they will be marketed. Only full-scale systems shall be tested. Vendors seeking treatment credits for filter series systems shall have their products tested in the series configuration.

This PROTOCOL and the QA/QC requirements contained herein meet the requirements of the EPA Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) (40 CFR §141.719), as described in the EPA LT2ESWTR Toolbox Guidance Manual (TGM), which in turn refers to the EPA Membrane Filtration Guidance Manual (MFGM) for the testing protocol requirements.

Under LT2ESWTR, individual bag or cartridge filters can receive *Cryptosporidium* treatment credit up to 2.0-log, while filters operated in series can receive credit up to 2.5-log (40 CFR §141.719(a)). The results of challenge tests following this generic ETV Protocol will allow States or other primacy agencies to award *Cryptosporidium* removal credits for bag and cartridge filtration systems.

1.0 Equipment Verification Testing Responsibilities

1.1 Verification Test Site

Testing shall be performed at a test facility/laboratory such that the testing equipment includes at minimum: injection pumps and ports to introduce the challenge microorganism, source of acceptable quality of water consistent with section 3.0 of MFGM, flow rate control and a flow meter upstream and/or downstream of the module; and ensure that the water is well mixed before sampling (e.g., static mixers or appropriate number of pipe lengths with good mixing confirmed).

1.2 Roles and Responsibilities

The roles and responsibilities of the testing facility, its credentials and experience with membrane testing, the vendor, and the EPA ETV Program shall be described in the Test Quality Assurance Plan (TQAP).

1.2.1 Testing Facility

Testing should be performed by an independent third party organization or laboratory. For testing under the Environmental Protection Agency (EPA) Environmental Technology Verification (ETV) Program, an independent third party performing testing and preparation of the ETV verification report shall conform to the EPA ETV Program's Quality Management Plan (QMP).

Independent third parties include those accredited by their respective nation's "accreditation bodies" to ISO Standards for independent third party testing organizations and certifiers: ISO Guide 65 and Standard 17025. ISO Guide 65 contains the general requirements for bodies operating product certification systems. ISO 17025 contains the general requirements for the competence of testing and calibration facilities and organizations.

All testing organizations and laboratories shall follow the procedures and adhere to the requirements of this PROTOCOL. The testing organization shall provide the following information in the ETV TQAP and ETV Report the following information:

- Test site description including schematic of hydraulics laboratory and geographic location of the facility;
- Key personnel with identified roles and responsibilities related to testing and their experience (resumes may be included or provided upon request);
- Present accreditations of the test facility such as conformance to ISO Standard 17025 or ETV QMP;
- Approximate schedule for testing.

1.2.3 United States Environmental Protection Agency

The EPA provides leadership in the nation's environmental science, research, education and assessment efforts. The EPA works closely with other federal agencies, state and local governments, and Native American tribes to develop and enforce regulations under existing environmental laws. The agency is responsible for researching and setting national standards for a variety of environmental programs and delegates to states and tribes responsible for issuing

permits, and monitoring and enforcing compliance. Where national standards are not met, the EPA can issue sanctions and take other steps to assist the states and tribes in reaching the desired levels of environmental quality. The Agency also works with industries and all levels of government in a wide variety of voluntary pollution prevention programs and energy conservation efforts.

The following are specific EPA roles and responsibilities:

- Quality Assurance (QA) oversight of ETV related activities;
- Technical review and QA oversight of the PROTOCOL;
- Technical and QA reviews and approval of a vendor product test/quality assurance plan (TQAP);
- Direct the performance, at the EPA's discretion, of external technical systems audit(s) during the verification testing;
- Review draft verification reports and statements; and
- Final report approval and clearance for signature by the EPA Laboratory Director.

2.0 Equipment Description

The vendor (equipment manufacturer) shall supply a complete description of the bag or cartridge system to be tested. The description shall include the following:

- Model name/number;
- Maximum design flow rate;
- Maximum inlet pressure;
- Terminal pressure drop requiring filter changeout;
- Exploded schematic diagram of the filter element and housing; and
- Status of module certification to NSF/ANSI Standard 61.

The vendor shall also submit a wetted parts list. For each wetted part/material, the list shall provide the part name and number, the material trade name or part trade name, the supplier, and the wetted surface area.

A minimum of two filter units shall be tested. A filter unit is defined in the EPA LT2ESWTR Toolbox Guidance Manual (TGM) as the filter media (bag or cartridge), housing, and associated piping and valves. The vendor may submit more than two units for testing if desired, or if required by a regulatory agency. The bags or cartridges to be tested should be selected from different production runs, if possible.

3.0 Experimental Design

3.1 Experimental Design

This PROTOCOL is adapted from the EPA LT2ESWTR TGM, the EPA MFGM, and the microbial seeding study in the EPA *Environmental Technology Verification Protocol for Equipment Verification Testing for Physical Removal of Microbiological and Particulate Contaminants*. The Environmental Technology Verification (ETV) Protocol is cited in the MFGM as an acceptable approach for challenge testing.

The vendor shall submit two or more units (single vessels, or multi-vessel configurations for serial filtration systems) for testing.

LT2ESWTR states that filters shall be tested at the maximum design flow rate for a duration sufficient to reach one hundred percent (100%) of the terminal pressure drop. Each filter tested shall be challenged with the challenge particulate within two hours of start-up of a new filter, when the pressure drop is between 45 and 55 percent of the terminal pressure drop, and after the terminal pressure drop has been reached (40 CFR §141.719).

3.2 Challenge Organisms

The bag and cartridge filter section of the TGM refers to the MFGM for the characteristics of acceptable surrogates for *Cryptosporidium*. For ultrafiltration module tests following the MFGM requirements, a case was made to stakeholders for using *Bacillus atrophaeus* endospores as a surrogate for *Cryptosporidium*. See Appendix A for further discussion and the rationale for *B. atrophaeus* endospores as a surrogate for *Cryptosporidium.* Stakeholders agreed with the use of *B. atrophaeus* endospores as a surrogate for *Cryptosporidium*, as presented in the ETV Protocol *Generic Protocol for Product-Specific Challenge Testing of Microfiltration or Ultrafiltration Membrane Modules*. Therefore, testing organizations may also use *B. atrophaeus* endospores as a *Cryptosporidium* surrogate for bag and cartridge filters.

B. atrophaeus was chosen because it yields orange colonies with a distinctive morphology on trypicase soy agar (TSA), so it can be distinguished from wild-type endospores that could be present as contamination. *B. atrophaeus* endospores are ellipsoidal (football shaped), with an average diameter of 0.8 µm, and an average length of 1.8 µm.

LT2ESWTR states that individual bag and cartridge filters are eligible for up to 2.0-log removal credit for *Cryptosporidium* if they demonstrate a minimum 3.0-log removal in challenge testing. Bag and cartridge filters in series are eligible for up to 2.5-log removal credit if they demonstrate a minimum 3.0-log removal. LT2 also states that the challenge particulate maximum feed concentration is 1.0×10^4 times the filtrate detection limit, to prevent overseeding leading to artificially high log removals. If a testing organization's filtrate detection limit for *B. atrophaeus* is 1 CFU/100 mL, then the maximum allowable feed concentration would be 1.0×10^4 CFU/100 mL. However, should the maximum allowable feed concentration accidentally exceed the target by 1.0-log or less, the testing organization may elect to not re-run the challenge test, but rather reset the feed concentration to 1.0×10^4 CFU/100 mL for the purpose of calculating log reductions.

3.3 Test Apparatus

The filters shall be tested in a test rig that conforms to the requirements of the MFGM. See Section 3.11.1 of the MFGM for example schematic diagrams of acceptable test rigs.

The challenge organisms shall be introduced into the feed water by intermittent injection. Injection and mixing of the organisms shall follow the guidelines of the MFGM. Specifically:

- the stock solution volume for injection shall be between 0.5 and 2 percent of the total estimated test solution volume;
- a chemical metering pump that delivers a steady flow of the challenge solution shall be used;
- the injection port shall include a quill that extends into the middle of the feed pipe;
- the test rig shall include an in-line static mixer in between the injection and feed sample ports;
- feed and filtrate grab samples shall be collected from sample ports that also have quills extending into the middle of the pipe;
- the sample taps shall be metal so they can be flame-sterilized prior to sample collection;
- the feed sample tap shall be located at least ten pipe diameters downstream of the injection point; and
- both the feed and filtrate sample taps shall be located as close as possible to the test unit.

3.4 Filter Operation

The filtration systems shall be operated at the vendor's maximum design flow. There is no requirement for inlet pressure; it shall be set as necessary to achieve the required flow. Each filter shall be tested for a duration sufficient to reach terminal pressure drop.

3.5 Test Water Composition

The test water shall conform to Section 8.4.1.5 of the TGM. The TGM calls for using a low to moderate concentration of suspended solids so the pressure drop does not build up too quickly to conduct the challenge tests. Two different water supplies are acceptable; one with a higher concentration of suspended solids to build up head loss, and a second water supply with low suspended solids for conducting the microbial challenges.

The test waters shall be analyzed for total chlorine, alkalinity, pH, temperature, total dissolved solids, total organic carbon, and turbidity.

3.5 Sanitizing the Test Rig

Prior to initiation of testing, and during each module change out, the test rig shall be sanitized using a bleach solution at an appropriate concentration and exposure time.

3.6 Filter Conditioning

Prior to initiation of testing, the filters shall be conditioned following a procedure supplied by the vendor.

3.7 Microbial Challenge Test Procedure

The challenge test procedure shall comply with the requirements of Sections 3.10, 3.11, and 3.12 of the MFGM. Each of the units submitted for testing shall be challenged individually. There shall be no conditioning period, other than that specified by the vendor to prepare the filters for service.

1. Each test unit shall be individually plumbed to the test rig after the rig has been sanitized and rinsed.
2. The filters shall be conditioned per the vendor's instructions. During this period the feed flow and inlet pressure shall be adjusted as necessary to obtain the proper flow for the challenge test. The flow shall be set at the vendor's maximum recommended value.
3. At the end of the conditioning period, a negative control filtrate sample shall be collected for challenge organism enumeration.
4. Filter operation shall begin at the proper flow. Injection of the challenge organism suspension shall be started. Feed and filtrate samples shall be collected after at least three void volumes of water containing the challenge organism have passed through the test unit, to allow for establishment of equilibrium. The vendor shall provide the unit void volume, or alternatively, the calculated approximate volume of the housing can be used to provide an additional safety factor. For instance, if the housing is a typical cylinder design, the calculated volume of a cylinder of the height and diameter of the housing, plus the volume of any piping. After the appropriate injection time, grab samples shall be collected from the feed and filtrate sample taps. The sample taps shall be flame sterilized, and then fully flushed prior to sample collection. After sample collection is complete, challenge suspension injection shall be stopped and filter operation shall continue.
5. The filter shall be operated until the pressure drop across the filter is 50 ± 5 percent of the terminal pressure drop value. At this point, the second microbial challenge shall be conducted following the procedure in Step 4.
6. Immediately following the second microbial challenge, resume filter operation until the terminal pressure drop is reached. Repeat Step 4 to conduct the final microbial challenge.
7. Immediately after the terminal pressure drop microbial challenge is complete, filter operation shall be stopped for five minutes, then restarted. Samples for *B. atrophaeus* analysis shall be collected from the first filtrate water out of the system upon restart, then again after five minutes of operation and ten minutes of operation. There shall be no injection of the endospores during this sampling period. The test is complete.

4.0 Laboratory Operations Procedures

4.1 Introduction

This protocol specifies procedures that shall be used to ensure accurate documentation of bag and cartridge filters. Careful adherence to these procedures and to the analytical procedures shall result in verifiable performance data.

4.2 Analytical Methods

A list of analytical methods is provided in Table 4.1.

Table 4.1 Analytical Methods for Laboratory Analyses

Parameter	Method
Alkalinity (total)	SM 2320B(1)
pH	SM 4500-H^+ B
TDS	SM 2540 C
Total Chlorine	SM 4500-Cl G
Turbidity	SM 2130 B
B. atrophaeus Endospores	SM9218(3)
C. parvum oocysts	EPA 1623

(1) SM = Standard Methods
(2) Immediate analysis required
(3) Trypticase soy agar (TSA) is substituted for nutrient agar in SM 9218 so that the challenge endospores can be distinguished from wild-type endospores. TSA gives orange colonies with a distinctive morphology.

4.3 Analytical QA/QC Procedures

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

- Alkalinity – A certified QC sample shall be analyzed each day. The acceptable recovery limit is that specified with the sample.
- pH – Three-point calibration (4, 7, and 10) of the pH meter used to give the reportable data shall be conducted daily using traceable buffers. The accuracy of the calibration shall be checked daily with an independent (separate lot, and preferabley separate source from the calibration buffers) buffer. The pH reading for the buffer shall be within 0.1 S.U. of its true value. The precision of the meter shall be checked daily using duplicate synthetic drinking water samples. The duplicate samples shall be within 0.1 S.U.
- TDS – A QC sample shall be analyzed with each sample batch. The percent recovery shall be within 10%, or the QC sample manufacturer's specified limits. Also, one blank (empty evaporating dish) is run with each batch, and shall be within 0.5 mg of original weight. Ten percent of samples shall be analyzed in duplicate, and should agree with 5% of average weight (10% RPD).
- Temperature – The thermometer used to give the reportable data shall have a scale marked for every 0.1ºC. The thermometer shall be calibrated yearly against a NIST-traceable reference thermometer.

- Total chlorine – The calibration of the chlorine meter shall be checked daily using a DI water sample (blank), and three QC standards. The measured QC standard values shall be within 10% of their true values. The precision of the meter shall be checked daily by duplicate analysis of synthetic drinking water samples. The RPD of the duplicate samples shall be less than 10%.
- Turbidity – The turbidimeter shall be calibrated as needed according to the manufacturer's instructions with formazin standards. Accuracy shall be checked daily with a secondary Gelex standard. The calibration check shall give readings within 5% of the true value. The precision of the meter shall be checked daily by duplicate analysis of synthetic drinking water samples. The RPD of the duplicate samples shall be less than 10%.
- Sample processing and enumeration of *B. subtilis* endospores.
    - Samples shall be stored in the dark at 3 ± 2 °C until analyzed.
    - All samples shall be analyzed in triplicate.
    - All batches of media shall be checked for sterility and for positive growth response.
    - Membrane filters and dilution water shall also be checked for sterility.

4.4 Sample Handling

All samples not immediately analyzed shall be labeled with unique identification numbers. These identification numbers shall be entered into a Laboratory Information Management System (LIMS), and shall appear on the lab reports for the tests. All challenge organism samples shall be stored in the dark at 4 ± 2 °C and processed for analysis within twenty-four hours or within the holding times listed in Table 4-1.

4.5 Quality Control Checks

4.5.1 Duplicate Samples

Duplicate samples shall be analyzed to determine the precision of analysis. Duplicate samples shall include both parameters measured immediately in the testing laboratory itself, and those submitted to a different laboratory group for analysis. For submitted samples, the laboratory shall analyze at least ten percent of samples in duplicate, and/or shall analyze a set of matrix spike duplicates with the sample batch. For parameters measured immediately in the testing laboratory, at least ten percent of samples shall be collected in duplicate for duplicate analysis.

4.5.2 Method Blanks and Spiked Samples

Method blanks and spiked samples shall be included, where appropriate, by the Chemistry Laboratory as part of the sample analysis methodology. The method blank and spiked sample requirements shall be addressed in the testing organization's Quality Assurance Manual.

4.5.3 Travel Blanks

In the event that samples are sent offsite for analysis, travel blanks shall accompany them, if appropriate, to check for any travel-related contamination. Should a testing organization collect samples in the same facility as where the samples are analyzed, there is no need for travel blanks.

4.5.4 Performance Evaluation Samples

Test organizations should routinely participate in performance evaluation studies. The performance evaluation requirements should be included in the Laboratory Quality Assurance Manual.

4.6 Flow Rate

During validation testing, the variability or precision of flow rate measurements should be less than or equal to five percent**.** The measurement uncertainty of the flow meter shall be verified by the catch and weigh method or for larger flow rates that cannot be "caught and weighed", by an alternative method, such as a calibrated pitometer or second calibrated flow meter, that can provide the required flow meter calibration and uncertainty data.

4.7 Documentation

All laboratory activities shall be thoroughly documented using lab bench sheets. Lab bench sheets shall be used to record all water treatment equipment operating data. Each page shall be labeled. Errors shall have one line drawn through them and this line shall be initialed and dated.

Any deviations from the approved final protocol shall be thoroughly documented at the time of inspection (see Section 5.5) and in the ETV report.

5.0 Quality Assurance Project Plan

5.1 Introduction

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

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

5.2 Quality Assurance Responsibilities

A number of individuals may be responsible for test equipment operation, sampling, and analysis QA/QC throughout verification testing. Primary responsibility for ensuring that these activities comply with the QA/QC requirements of this TQAP rests with the supervisors of the individuals. Laboratory QA/QC staff shall review the raw data records for compliance with QA/QC requirements. Staff shall check 100% of the raw data records against the reported results in the laboratory information management system (LIMS) reports.

5.3 Data Quality Indicators

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

5.3.1 Representativeness

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

5.3.2 Accuracy

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

Accuracy shall be maintained through the following means:

- Maintaining consistent sample collection procedures, including sample locations, timing of sample collection, and sampling procedures;
- Calibrated instruments; and

- Laboratory control samples (e.g., method blanks, duplicates, matrix spikes, matrix spike duplicates, and performance evaluation samples).

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

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

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

Recoveries for laboratory control samples are calculated as follows:

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

For acceptable analytical accuracy, the recoveries must be within control limits specified in Section 4.3. The accuracy of the benchtop chlorine, pH, and turbidity meters shall be checked daily during the calibration procedures using certified check standards. For samples analyzed in batches certified QC samples shall be run with each batch.

5.3.3 Precision

Precision refers to the degree of mutual agreement among individual measurements and provides an estimate of random error. Precision shall be measured through duplicate sample analysis. One sample per batch shall be analyzed in duplicate for the TDS and alkalinity analyses. To check the precision of the benchtop chlorine, pH, and turbidity meters, duplicate synthetic drinking water samples shall be analyzed daily. Precision of the duplicate analyses in which the total number of samples is fewer than eight, the Relative Percent Difference (RPD) shall be measured by use of the following equation:

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

where:

S_1 = sample analysis result; and

S_2 = sample duplicate analysis result.

Acceptable RPD values for each parameter are given in Section 4.3.

The use of percent relative standard deviation may be used if the number of samples is eight or greater. Percent relative standard deviation shall be calculated as follows:

$$\% \text{ Relative Standard Deviation} = \frac{S(100)}{X_{average}}$$

where: S = standard deviation
$X_{average}$ = the arithmetic mean of the recovery values.

Standard Deviation is calculated as follows:

$$\text{Standard Deviation} = \sqrt{\frac{\sum_{i=1}^{n}(X_i - X)^2}{n-1}}$$

where: X_i = the individual recovery values
X = the arithmetic mean of then recovery values
n = the number of determinations.

For acceptable analytical precision under the verification testing program, the percent relative standard deviation for drinking water samples must be less than 30%.

5.3.4 Statistical Uncertainty

Statistical uncertainty of the triplicate challenge organism counts shall be evaluated to calculate the 95% confidence intervals. The following formula shall be employed for confidence interval calculation:

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

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

5.3.5 Completeness

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

The completeness objective for data generated during verification testing is based on the number of samples collected and analyzed for each parameter and/or method. The following chart illustrates the completeness objectives for performance parameter and/or method based on the sample frequency:

Number of Samples per Parameter and/or Method	Percent Completeness
0-10	80%
11-50	90%
>50	95%

Completeness is defined as follows for all measurements:

%C = (V/T) X 100

where:

%C = percent completeness;
V = number of measurements judged valid;
T = total number of measurements.

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

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

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

5.4 Data Validation and Reporting

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

5.4.1 Data Validation

For the analytical data:

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

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

5.4.2 Data Reporting

The data to be reported shall be the feed and treated water microorganism counts, log reductions, and the water chemistry data. All bench sheets and QA/QC analyses shall be included with the report as an appendix.

5.5 Testing Inspections

The test facility QA department shall conduct an audit of the laboratory during testing to ensure compliance with the test procedures and requirements of this protocol. The results of all such internal audits shall be reported to the laboratory staff. Throughout testing, staff shall carry out random spot inspections. Any variances shall be reported to QA department.

6.0 Data Management, Analysis, and Reporting

6.1 Data Management and Analysis

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

6.2 Work Plan

The following is the work plan for data management:

- Laboratory personnel shall record equipment operation, water quality and analytical data by hand on bench sheets.
- All bench sheet entries shall be made in water-insoluble ink.
- All corrections on the bench sheets shall be made by placing one line through the erroneous information. Any corrections shall be dated and initialed by the lab personnel making the correction.
- Pertinent information from the bench sheets shall be entered into a laboratory information management system or equivalent.

The database for verification testing programs shall be set up in the form of custom-designed spreadsheets. Pertinent lab data shall be entered into the appropriate spreadsheets. All recorded calculations shall also be checked at this time. Following data entry, the spreadsheet shall be printed out and the printout checked against the official laboratory data reports or bench sheets.

6.3 Performance Reporting

Microorganism removal shall be evaluated through log reduction calculations. All challenge organism samples shall be analyzed in triplicate, and geometric means calculated. The geometric means shall be log transformed for the purpose of calculating log reductions. To calculate average log reductions, the *arithmetic* means of the logs of the individual sampling points shall be calculated.

6.4 Report of Equipment Testing

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

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

Appendix A
Bacillus Endospores as a Surrogate for *C. parvum* Oocysts

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

1. Organism Size and Shape

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

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

2. Electrophoretic Mobility and Isoelectric Point

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

Lytle et. al. (2002) measured the EPM of both *C. parvum* and *B. subtilis* endospores in solutions of increasing buffer concentration (0.915 millimolar, mM, 9.15 mM, and 91.5 mM KH_2PO_4). They found that increasing the buffer concentration also increases the EPM toward a positive value. The buffer concentration of the test water for the Siemens tests was approximately 1 mM. Therefore, the 0.915 mM data from this study should be the most accurate representation of the

C. parvum and *B. subtilis* EPM for the ETV tests. In 0.915 mM solutions at pH values between 7 and 8, they observed EPM of approximately -2.2 to -2.6 µm cm V^{-1} s^{-1} for *C. parvum*, and -1.9 to -2.2 µm cm $V^{-1}s^{-1}$ for *B. subtilis*. For *B. subtilis*, the researchers did not measure an isoelectric point at any buffer concentration. For *C. parvum*, they did find an isoelectric point at a pH around 2.5, but only for the 9.15 mM solution. For both organisms, the 0.915 mM solution generally gave lower (more negative) EPM values than the solutions with higher buffering capacity.

3. Aggregation

The NSF Microbiology Laboratory microscopically examined a sample of the *B. atrophaeus* stock solutions purchased for the tests. The sample was suspended in sterile, buffered, deionized water and stirred at moderate speed for 15 minutes. The estimated cell density was $1x10^9$ CFU/100 mL, which is approximately 100 times higher than the suspensions injected into the pilot units to challenge the UF membranes. Figure 1 is a photograph of the *B. atrophaeus* endospores in the sample. The magnification is 1000x oil immersion with differential interference contrast microscopy. No evidence of endospore aggregation was found.

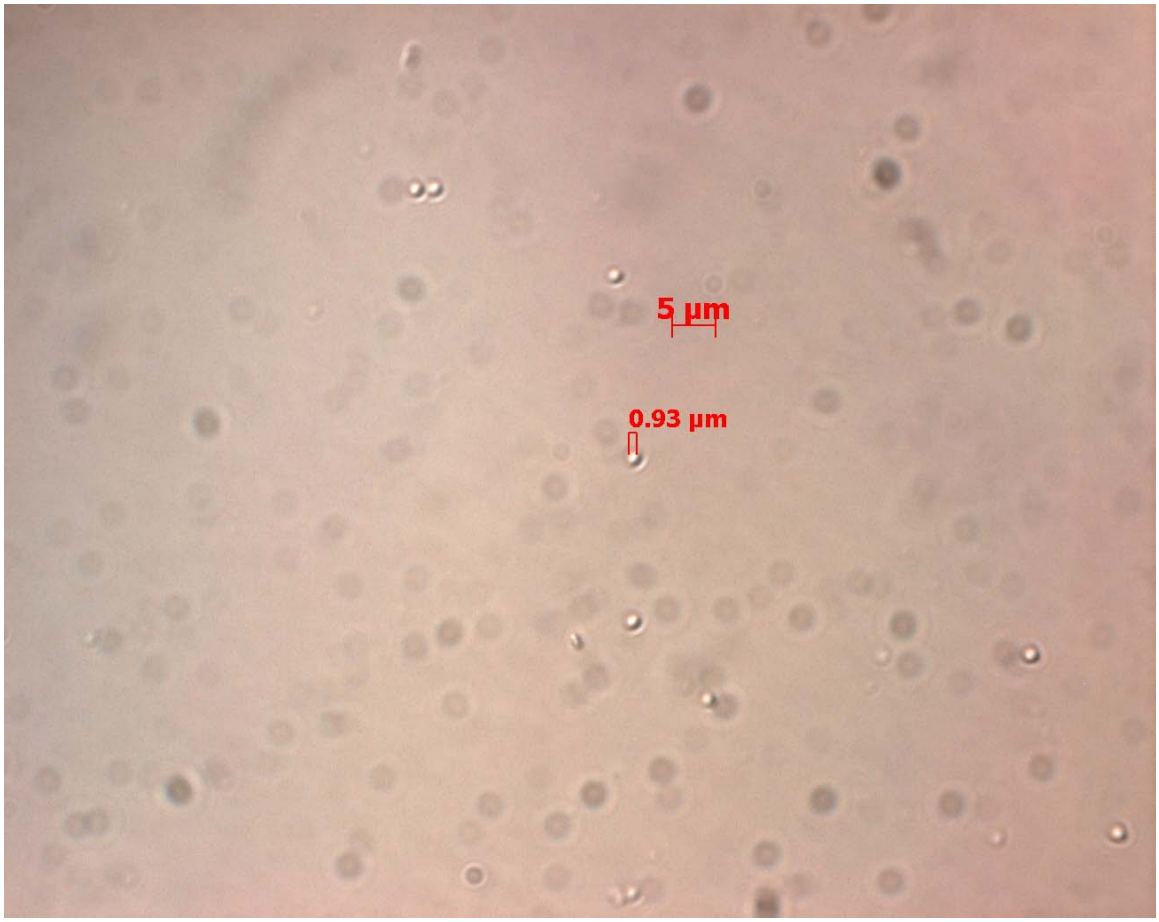

Figure B-1. Mono-dispersed *B. atrophaeus* endospores used for challenge tests.

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