MEMORANDUM

TO: Joint Committee on Public Drinking Water Equipment Performance

FROM: Robert Powitz, Chairperson

DATE: October 23, 2014

SUBJECT: Proposed draft standard NSF 419 – *Public Drinking Water Equipment Performance – Filtration* (419i1r3).

Draft 3 of NSF 419 issue 1, is being forwarded to the Joint Committee for balloting. Please review the proposed standard and **submit your ballot by November 13, 2014** via the NSF Online Workspace.

When adding comments, please identify the section number/name for your comment and add all comments under one comment number where possible. If you need additional space, please upload a word or pdf version of your comments online via the browse function.

Purpose

It is the purpose of this standard to establish minimum performance requirements for membrane filtration devices used in the treatment and production of public drinking water. It includes the test procedures for product specific challenge testing of full scale UF and MF membrane modules, bag filters, and cartridge filters for the removal of microbial contaminants. This standard provides testing procedures to develop Log Removal Values (LRV_{C_TEST}), as required in the EPA's Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR). Please note that a foreword will be included with the final published standard which will provide additional background information and address the potential for other technologies (including RO systems) to be included under this standard in the future.

Background

At the end of 2013, the EPA Environmental Technology Verification (ETV) Program ended and stakeholders were no longer able to update the ETV protocols addressing full scale UF and MF membrane modules, bag filters, and cartridge filters for the removal of microbial contaminants. NSF 419, which is closely derived from these protocols, will establish minimum performance requirements for these devices and specify test procedures for product specific challenge testing.

An initial meeting was held on July 17, 2013 by the Joint Committee on Public Drinking Water Equipment Performance to review the proposed draft standard. A straw ballot was then sent to the committee and a follow-up meeting took place on September 3, 2014 to discuss comments received during the initial straw ballot. **Revision 3** incorporates

recommendations made by the JC during the straw ballot and subsequent meeting discussion.

A reference document showing the changes made to the previous straw ballot draft (revision 2) has been included under the reference documents to assist you in your review. Please also find the 2013 and 2014 PDWEP JC meeting summaries attached under the reference documents for additional information.

If you have any questions about the technical content of the ballot, you may contact me in care of:

Chairperson, Joint Committee
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NSF Standard
for Public Drinking Water Equipment Performance -

Public Drinking Water Equipment Performance – Filtration

1 General

1.1 Purpose

It is the purpose of this Standard to establish minimum performance requirements filtration devices used in the treatment and production of public drinking water.

1.2 Scope

This standard is designed to describe the performance evaluation test procedure for the product specific challenge testing of full scale UF and MF membrane modules, bag filters, and cartridge filters for the removal of microbial contaminants. This standard provides procedures to develop challenge testing Log Removal Values (LRV_{C_TEST}), as required in the EPA's Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) published in 40 CFR 141-subpart W.

Evaluation of cleaning, maintenance and operation of the filtration equipment are not covered under the scope of this Standard.

1.3 Alternate materials, designs, and construction

While specific materials, designs, and construction are stipulated in this Standard, it is possible that systems that incorporate alternate materials, designs, and construction are acceptable when it is verified that such systems meet the applicable requirements stated herein.

1. 4 Minimum requirements for testing facility and equipment

Testing should be performed at a test facility/laboratory such that the testing equipment at a minimum shall precisely and accurately control flow rate and has a flow meter upstream and/or downstream of the filter unit or membrane module; and shall ensure that the water is well mixed before sampling (e.g., static mixers or appropriate number of pipe lengths with good mixing confirmed).

1.5 Standard review

This Standard shall be reviewed at least once every five years. The review shall be conducted by the NSF Joint Committee on Public Drinking Water Equipment Performance.

1.6 Significant figures

For determining conformance with specifications in this Standard, the Absolute Method in ASTM E29 *Standard Practice for Using Significant Digits in Test Data to Determine Conformance with Specifications* shall be used.

2 Normative References

The following documents contain provisions that constitute requirements of the Standard. At the time of the publication, the indicated editions were valid. All standards are subject to revision, and parties are encouraged to investigate the possibility of applying the recent editions of the standards indicated below. The most recent published edition of the document shall be used for undated references.

40 CFR Part 141.719, *National Primary Drinking Water Regulations; Additional filtration toolbox components*[1]

APHA, *Standard Methods for the Examination of Water and Wastewater*, twentieth edition[2]

ASTM D6908-03 *Standard Practice for Integrity Testing of Water Filtration Membrane Systems*[3]

ASTM E29-02 *Standard Practice for Using Significant Digits in Test Data to Determine Conformance with Specifications*[3]

NSF/ANSI 61 – *Drinking Water System Components – Health Effects*.

3 Definitions

The following terms are used in this document, and were derived from the definitions in the EPA guidance manuals for LT2ESWTR referenced herein.

3.1 bag and cartridge filters: Pressure driven separation devices that remove particles using an engineered porous filtration media.

3.2 challenge particulate: The target organism or acceptable surrogate used to determine the log removal value (LRV) during a challenge test.

3.3 crossflow: 1) The application of water at high velocity tangential to the surface of a membrane to maintain contaminants in suspension; also, 2) suspension mode hydraulic configuration that is typically associated with spiral-wound nanofiltration (NF) and reverse osmosis (RO) systems and a few hollow-fiber microfiltration (MF) and ultrafiltration (UF) systems.

3.4 deposition mode: A hydraulic configuration of membrane filtration systems in which contaminants removed from the feed water accumulate at the membrane surface (and in microfiltration (MF)/ultrafiltration (UF) systems are subsequently removed via backwashing).

3.5 direct integrity test: A physical test applied to a membrane unit in order to identify and/or isolate integrity breaches.

[1] Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402 www.gpo.gov
[2] American Public Health Association (APHA), 800 I Street, NW, Washington, DC 20001 <www.apha.org>
[3] ASTM International, 100 Barr Harbor Drive, West Conshocken, PA 19428-2859 www.astm.org.

3.6 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).

3.7 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)).

3.8 hydraulic configuration: The pattern of flow through a membrane process by which the feed contaminants are removed or concentrated (e.g., crossflow, dead-end, etc.)

3.9 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)).

3.10 membrane unit: A group of membrane modules that share common valving which allows the unit to be isolated from the rest of the system for the purpose of integrity testing or other maintenance.

3.11 microfiltration (MF): A pressure-driven membrane filtration process that typically employs hollow-fiber membranes with a pore size range of approximately 0.1 – 0.2 mm (nominally 0.1 mm).

3.12 module: The smallest component of a membrane unit in which a specific membrane surface area is housed in a device with a filtrate outlet structure; refers to all types of membrane configurations, including terms such as “element” or “cartridge” that are commonly used in the membrane treatment industry.

3.13 non-destructive performance test (NDPT): A physical quality control test typically conducted by a manufacturer to characterize some aspect of process performance without damaging or altering the membrane or membrane module.

3.14 quality control release value (QCRV): A minimum quality standard of a non-destructive performance test (NDPT) established by the manufacturer for membrane module production that ensures that the module will attain the targeted log removal value (LRV) demonstrated during challenge testing in compliance with the LT2ESWTR.

3.15 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.

3.16 ultrafiltration (UF): A pressure-driven membrane filtration process that typically employs hollow-fiber membranes with a pore size range of approximately 0.01 – 0.05 mm (nominally 0.01 mm).

4 Materials

Materials for filtration devices shall conform to the requirements of NSF/ANSI 61.

5 Bag and cartridge filter systems

5.1 General requirements

5.1.1 A complete description of the bag or cartridge system to be tested shall be provided. The description shall include the following information:

- model name/number of cartridge/bag and filter vessel;
- 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 61.

5.1. 2 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. More than two units are permitted to be tested if required by a regulatory agency. The bags or cartridges to be tested should be selected from different production runs, if possible.

5.2 Experimental design

5.2.1 The two units shall be configured in parallel for testing or multiple vessel units should be configured for testing in series.

5.2.2 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.

5.3 Challenge particulate

5.3.1 The system shall be tested using polystyrene latex microspheres. The polystyrene microspheres shall have 95% of particles in the range of 3.00 ± 0.15 µm. The size variation of the polystyrene microspheres shall be confirmed by electron microscopy. The spheres shall have a surface charge content of less than 2 uEq/g. The microspheres shall contain a fluorescein isothiocyanate (FITC) dye or equivalent.

5.3.2 The maximum feed concentration shall be 1.0×10^4 times the filtrate detection limit, to prevent overseeding leading to artificially high log removals.

5.4 Apparatus

The filters shall be tested in a test apparatus that meets the requirements of LT2ESWTR and the objectives of this standard and its scope. At a minimum, a test apparatus suitable for conducting challenge testing should include equipment such as pumps, valves, instrumentation, and controls necessary to evaluate full-scale modules. The test apparatus should also be designed to mimic the hydraulic configuration of the full-scale system as much as practical. The test equipment should be capable of providing the precision and accuracy necessary to generate data within the requirements of this Standard.

5.5 Flow rate

The filtration systems shall be operated at the manufacturer's specified 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.

5.6 General Test Water

A dechlorinated, potable water supply shall be used with the following characteristics:

alkalinity	≥ 20 mg/L
HPC	< 500 bacterial colonies/mL
iron[1]	< 0.3; recommend non-detectable levels
manganese[1]	< 0.3; recommend non-detectable levels
pH	6.5-8.5
residual disinfection or oxidants in tap water (e.g., free chlorine, total chlorine, potassium permanganate, and chloramines)	None detected
temperature	10 - 27 °C (50 - 81 °F)
total organic carbon (TOC)[1]	Measure and report values in test report
turbidity	<0.3 NTU
[1]The levels of these parameters and any others present in the test water shall not be of a type and quantity to form a cake on the filtration media that could bias the observed reduction of challenge microspheres over the performance of the test.	

5.6.1 Test dust loading water

Test dust as required by the LT2ESWTR shall be added to the general test water specified in 5.6 to achieve a maximum of 10 NTU. The test dust shall have a nominal 0 to 5 µm size classification and shall have 96% (by volume percent) of its particles within this range and 20 to 40% (by volume percent) of its particles greater than 2.5 µm.

5.7 Set-up

5.7.1 Sanitization

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. A sample shall be collected to confirm that there is no microbiological contamination as defined in Annex B.

5.7.2 Conditioning

The filter units shall be conditioned in accordance with the manufacturer's instructions using the general test water specified in 5.6. If no conditioning instructions are provided, the units shall be flushed with a minimum of 3 hold-up volumes using the general test water specified in 5.6.

5.8 Method

There shall be no conditioning period, other than that specified by the manufacturer 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 section 5.7.2. During this period the feed flow and inlet pressure shall be adjusted as necessary to obtain the proper flow for the challenge test per section 5.5 of this Standard.
3. At the end of the conditioning period, a negative control filtrate sample shall be collected for challenge microsphere enumeration.
4. Filter operation shall begin at the proper flow. Injection of the challenge microsphere suspension shall be started. Feed and filtrate samples shall be collected after at least three void volumes of water containing the challenge microspheres 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 should 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 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 microsphere challenge shall be conducted following the procedure in Step 4.
6. Immediately following the second microsphere challenge, resume filter operation until the terminal pressure drop is reached. Repeat Step 4 to conduct the terminal pressure drop microsphere challenge.
7. Immediately after the terminal pressure drop microsphere challenge is complete, filter operation shall be stopped for a five minutes rest period, Operation shall then be restarted and injection of microspheres resumed. Samples for polystyrene microsphere 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.
8. LRV values shall be calculated according to the guidelines established in Annex C.

5.9 Analytical methods

This Standard 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.

5.9.1 Detection and enumeration of polystyrene microspheres shall be done in accordance with Annex A.

5.9.2 A list of analytical methods is provided in Table 1.

5.10 A final report shall be completed per guidelines established in Annex C.

Table 1- Analytical Methods for Laboratory Analyses

Parameter	**Method**[1]
Alkalinity (total)	SM 2320B
HPC	SM 9125
Iron	SM 200.7
Manganese	SM 200.7
pH	SM 4500-H^+ B
TDS	SM 2540 C
TOC	SM 5310B
Total Chlorine	SM 4500-Cl G
Turbidity	SM 2130 B

[1] *Standard Methods for the Examination of Water and Wastewater*

5.9.2 Flow rate

During validation testing, the variability or precision of flow rate measurements should be less than or equal to five percent*.*

6 Microfiltration and ultrafiltration membrane modules

6.1 General requirements

6.1.1 A complete description of the microfiltration or ultrafiltration membrane module to be tested shall be provided. The description shall include the following:

- vendor name;
- model name/number of membrane element and vessel (if applicable);
- membrane material;
- mode of operation (cross-flow, dead-end, or either; pressure or vacuum driven);
- type of membrane module configuration (e.g. hollow fiber, spiral wound, etc.);
- water flow through membrane (inside-out or outside-in);
- status of module certification to NSF/ANSI Standard 61, or equivalent; and
- the membrane specifications listed in Table 2.

Table 2 - Membrane Module Specifications

Dimensions:
Module outside diameter
Module length
Module volume - volume of pressurized air in module (volume of system)
Nominal and maximum membrane pore size, or molecular weight cutoff rating
Membrane surface area (feed side)
Operating Limits:
Max design Filtrate flux at 20°C
Flow range
Maximum inlet module pressure
Maximum transmembrane pressure (TMP)
Maximum oxidant tolerance

If the mode of operation is cross-flow, the vendor shall provide the maximum recommended recovery, so that testing is able to be conducted at the maximum volumetric concentration factor (VCF).

6.1.2 A minimum of five modules shall be tested, and greater than five is recommended. The modules should be selected by the filter manufacturer from five different production runs if possible.

6.2 Challenge Organisms

6.2.1 *B. atrophaeus* endospores shall be used as the surrogate for *Cryptosporidium* for testing membrane modules. For virus product specific challenge testing (PSCT), modules shall be challenged with the MS-2 coliphage virus. It is permissible for MS-2 coliphage to be used as a conservative *Cryptosporidium* surrogate.

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

- MS-2 – $5x10^5$ to $3.16 x10^6$ plaque forming units per milliliter (PFU/mL);
- *B. atrophaeus* – $5x10^5$ to $3.16 x10^6$ colony forming units (CFU) per 100mL; and

NOTE - The MFGM calls for the maximum challenge concentration to be 6.5 $\log_{10}$ above the organism's detection limit ($3.16x10^6$). The goal for the *B. atrophaeus* challenges is to be able to measure log reductions as close to 6.0 $\log_{10}$ without exceeding 6.5 $\log_{10}$.

6.3 Apparatus

The filters shall be tested in a test apparatus that meets the requirements of LT2ESWTR and the objectives of this standard and its scope. At a minimum, a test apparatus suitable for conducting challenge testing should include equipment such as pumps, valves, instrumentation, and controls necessary to evaluate full-scale modules. The test apparatus should also be designed to mimic the hydraulic configuration of the full-scale system as much as practical; however, it is permissible for the test apparatus to utilize a more conservative recovery (i.e. hydraulic efficiency) than the full-scale system. The test apparatus should allow the membrane module to undergo direct integrity testing both before and after the challenge test. The test equipment should be capable of providing the precision and accuracy necessary to generate data within the requirements of this Standard.

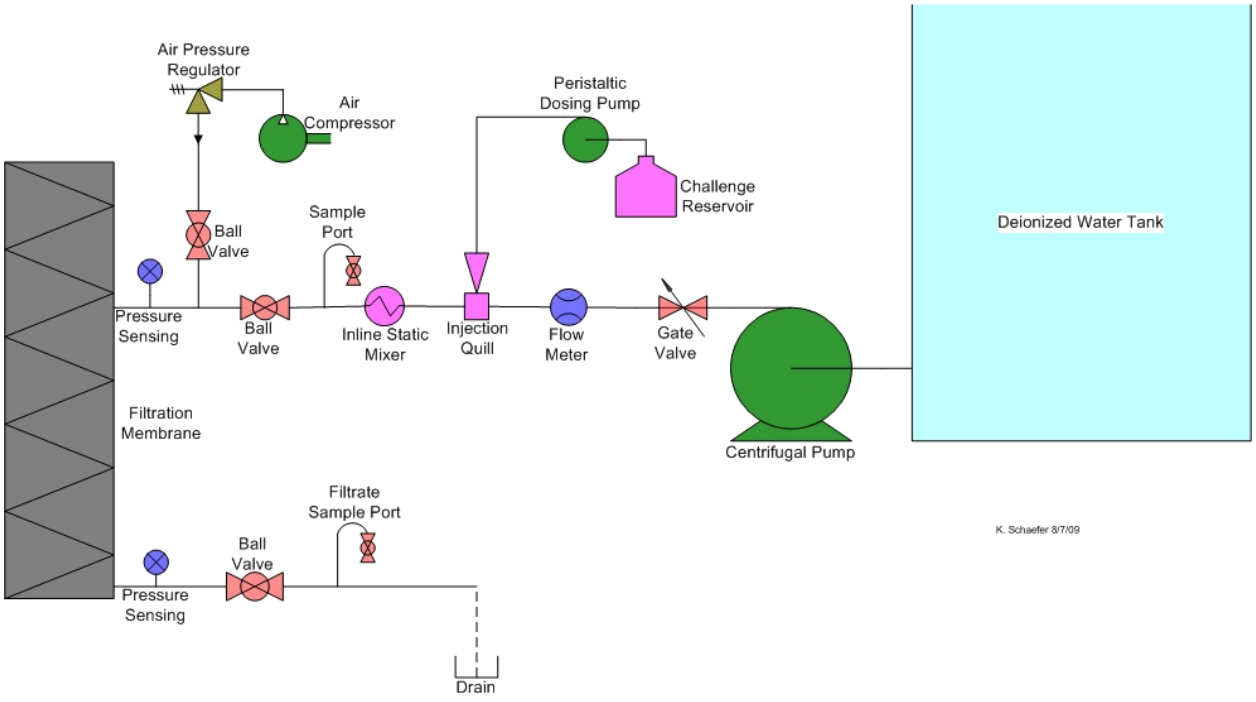

Figure 3 - Example test apparatus

The challenge organisms shall be introduced into the feed water by intermittent injection during the challenge tests. The stock solution volume for injection shall be between 0.5 and 2.0 percent of the total spiked test solution volume, a chemical metering pump that delivers a steady flow of the challenge solution shall be used, and the injection port shall include a quill that extends into the middle of the feed pipe. Proper mixing should be done to ensure that the influent solution is homogenous. If batch seeding is used, the feed concentration should not vary significantly over the course of challenge testing.

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

6.4 Flow rate

The modules shall be tested at the manufacturer's maximum design flux and maximum recovery. If the manufacturer markets the module to be used before drinking water operation in both cross-flow and dead-end mode, testing shall be conducted in cross-flow mode at the maximum recovery. The LRV measured in cross-flow mode should be also applied to the same module operating in dead-end mode, provided that the maximum design flux for the dead-end mode does not exceed the tested flux.

6.5 General Test Water

A dechlorinated, potable water supply shall be used with the following characteristics:

alkalinity	≥ 20 mg/L
HPC	< 500 bacterial colonies/mL
iron[1]	Recommended non-detectable levels and < 0.3 mg/L
manganese[1]	Recommended non-detectable levels and < 0.53 mg/L
pH	6.5-8.5
residual disinfection or oxidants in tap water (e.g., free chlorine, total chlorine, potassium permanganate, and chloramines)	None detected
temperature	10 - 27 °C (50 - 81 °F)
total organic carbon (TOC)[1]	Measure and report values in test report
turbidity	<0.3 NTU
[1]The levels of these parameters and any others present in the test water shall not be of a type and quantity to form a cake on the filtration media that could bias the observed reduction of challenge organisms over the performance of the test.	

A test organism viability and stability evaluation should be conducted as described in Annex B to determine if the test water is appropriate for the evaluation.

6.6 Set-up

6.6.1 Sanitization

Prior to initiation of testing, and during each module change out, the test rig shall be sanitized an appropriate disinfection.

6.6.2 Conditioning

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

6.7 Membrane Integrity Tests

Prior to testing, each module shall be subject to the same non-destructive performance test (NDPT) that the manufacturer uses at the production facility for quality control testing of each module manufactured. The results of this should be used to reset the manufacturing quality control release value (QCRV). Immediately before and after each individual module challenge test, the module shall undergo the manufacturer's recommended daily direct integrity test (DIT) for modules in-use.

A manufacturer's procedure for conducting a NDPT shall ensure that the QCRV associated with the minimal result from the NDPT, is indicative of a NSDPT resolution of 3 µm. Thus the NDPT shall be responsive to an integrity breach on the order of 3 µm or less (40 CFR 141.719 (b)(3)(ii)). The methods to determine the 3 µm resolution shall be done as described in section 4.2 Test Resolution of the Membrane Filtration Guidance Manual or in ASTM Method D 6908-03: *Standard Practice for Integrity Testing of Water Filtration Membrane Systems.*

6.8 Method

Each of the modules shall be challenged individually, and separate challenge tests shall be conducted for each challenge organism. The modules shall not have been used previously when challenged. There shall be no seasoning period, other than that specified by the vendor to sufficiently rinse out the membrane preservative and wet the membranes.

Each membrane shall be individually plumbed to the test rig after the rig has been sanitized and rinsed. If it is the first time the module is installed, it shall be flushed per the vendor's flushing and conditioning procedure. If the module has already been tested with another challenge organism, the module shall only be backwashed following a procedure supplied by the vendor, then forward flushed for at least five minutes at the test flow rate.

Following the forward flush, the pre-test DIT described in 6.7 shall be conducted.

After completion of the DIT, the module shall again be forward flushed for at least five minutes using the test water (minus challenge organism injection) specified in 6.5. After five minutes of flushing, two feed samples and two filtrate samples shall be collected. One sample from each process stream shall serve as a negative control, and shall be enumerated the challenge organism. The second sample pair shall serve as positive controls, and shall be spiked with the challenge organism.

During the forward flush, the feed flow, and also the reject flow if necessary, shall be adjusted to reach the proper flows for the challenge test.

Each challenge test shall be approximately 35 minutes in length. The challenge organism shall be injected into the feed stream at start-up, after 15 minutes of operation, and after 30 minutes of operation. As required in 6.3, the challenge organisms shall be intermittently injected into the feed stream prior to,

and during sample collection. The feed and filtrate samples shall not be collected until at least three hold-up volumes of water containing the challenge organism have passed through the membrane, to allow for establishment of equilibrium (equilibrium volume). The hold-up volume is defined as the "unfiltered test solution volume that would remain in the system on the feed side of the membrane at the end of the test." The vendor shall provide the module hold-up volume, or alternatively, the volume of the entire module shall be used to provide an additional safety factor. 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, the challenge suspension injection shall be stopped and clean test water shall be pumped through the modules until the next sampling point.

Log reduction values (LRV) shall be calculated, and a test report created using the guidelines provided in Annex C.

6.9 Analytical methods

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

6.9.1 A list of laboratory analytical methods for all parameters but MS-2 enumeration are found in Table 3. The analytical method for MS-2 is explained below the table.

Table 3 - Analytical methods for laboratory analyses

Parameter	Method
Alkalinity (total)	SM 2320B[1]
HPC	SM 9125
Iron	SM 200.7
Manganese	SM 200.7
pH	SM 4500-H^+ B
TDS	SM 2540 C
TOC	SM 5310B
Total Chlorine	SM 4500-Cl G
Turbidity	SM 2130 B
MS-2	See Section 6.9.3
B. atrophaeus Endospores	SM9218[2]

[1] *Standard Methods for the Examination of Water and Wastewater*
[2] Trypticase soy agar (TSA) shall be substituted for nutrient agar in SM 9218 so that the challenge endospores could be distinguished from wild-type endospores. TSA gives orange colonies with a distinctive morphology.

6.9.2 Sample processing and enumeration of MS-2 coliphages

One milliliter volumes of the feed samples shall be serially diluted for enumeration. One milliliter volumes of the filtrate samples shall be both enumerated directly and serially diluted for enumeration. The one mL volumes shall be added to tubes containing the host *E. coli* in tryptic soy broth (TSB). The tube shall be vortexed for 30 seconds, and then 4 mL of molten, tempered 1% tryptic soy agar (TSA) shall be added to

the tube. This mixture shall then be poured over a TSA plate, and the plate is incubated at 35 °C for 18-24 hours. The viral plaques shall be counted using a Colony Counter.

6.9.3 Sample handling

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 3.

6.9.4 Test controls

A Quality Assurance Project Plan as described in Annex B should be developed prior to testing and adhered to throughout the test. In addition, the following quality-control samples and tests shall be performed for each module tested:

- *Module blanks* – influent and effluent water samples shall be collected with no addition of challenge microorganism to the flow passing through the module. Blanks shall be collected for each module. The concentration of challenge microorganisms shall be quantified. Should the challenge microorganism exceed 0.2 $\log_{10}$ concentration, the plumbing shall be disinfected. Another reactor blank sample shall be collected and enumerated after disinfection and neutralization of the disinfectant. The reactor blank is considered acceptable for challenge testing if the challenge organism does not exceed 0.2 $\log_{10}$ concentration .

- *Trip controls* – one sample bottle of challenge microorganism stock solution shall travel with the stock solution used for validation testing from the microbiological laboratory to the testing location, and back to the laboratory. The change in the log concentration of the challenge microorganism in the trip control should be within the measurement error of 0.2 $\log_{10}$ concentration.

- M*ethod blanks* – a sample bottle of sterilized reagent grade water shall undergo the challenge microorganism assay procedure. No challenge microorganisms shall be detected in the method blanks.

6.9.5 Flow rate

During validation testing, the variability or precision of flow rate measurements should be less than or equal to five percent*.*

Annex A
(normative)

Test method for detecting and enumerating polystyrene microspheres

A.1 Summary of method

A 1-L sample shall be collected and an appropriate volume shall be passed through a membrane. The fluorescent microspheres deposited on the membrane shall be counted by scanning the membrane under an epifluorescence microscope.

In recognition of advances that are occurring in analytical technology, certain options shall be permitted to improve detection or lower the costs of measurements, provided that all quality control acceptance criteria are met. If an analytical technique other than the techniques specified in this method is used, that technique shall have a specificity equal to or better than the specificity of the techniques in this method for microspheres in the sample of interest. Specificity shall be defined as producing results that are equivalent to the results produced by this method for microspheres in drinking water and that meet all of the quality control (QC) acceptance criteria stated in this method.

A.2 Equipment

– fluorescent microspheres with fluorescein isothiocyanate (FITC) dye or equivalent (3 µm diameter);

– epifluorescence microscope with filters for FITC dye, 200x and 400x magnification;

– 0.45 µm 25 mm membrane filter;

– forceps;

– vacuum filtration apparatus;

– 1,000 mL glass separation funnel;

– autopipettes to dispense 0.10, 1.0, and 10.0 mL accurately;

– 75 mm x 50 mm glass slides;

– 1-L plastic sample bottles with caps;

– nail polish;

– hemocytometer chamber; and

– 10-place filter with manifold collection box and stainless steel wells.

A.3 Reagents

– polyoxyethylene sorbitan mono-oleate.

A.4 Enumeration of stock microspheres

A.4.1 Procedure using well slides

1) The stock microsphere suspension shall be vortexed, and 10 µL of an appropriate dilution (80 to 120 microspheres) shall be applied to all wells.

2) The wells shall be dried at 42 °C (108 °F) for 1 to 2 h.

3) The wells shall be mounted with 10 µL of DABCO-glycerol mounting medium.

4) The slides shall be enumerated and the concentration of the stock suspension shall be determined using the mean counts from the slides.

A.5 Procedure

A.5.1 Sample collection

Influent samples shall be collected in 1-L bottles containing 1 mL 1.0% polyoxyethylene sorbitan mono-oleate solution as a dispersant. The sample shall be refrigerated before filtering to prevent any bacterial growth. Influent samples shall be collected in triplicate.

3 L of the effluent shall be collected. The first liter of effluent shall be used as the test sample. The test samples shall be collected in 1-L bottles containing 1 mL 1.0% polyoxyethylene sorbitan mono-oleate solution as a dispersant. The sample shall be refrigerated before filtering to prevent any bacterial growth. The second and third liters of effluent shall be used for quality control samples. The second and third liters of effluent shall be composited and poured into two 1-L bottles each containing 1 mL 1.0% polyoxyethylene sorbitan mono-oleate and shall be refrigerated until analyzed.

The samples shall be prepared within 5 d of collection.

A.5.2 Filtration manifold preparation

The filtration manifold assembly shall be prepared by referring to the manufacturer's diagrams and instructions. The filtration manifold shall be connected to the vacuum supply using a vacuum tube containing a T-shaped tubing connector. A screw clamp shall be attached to 4 to 6 cm of latex tubing, and the latex tubing shall be attached to the stem of the "T" connector. The screw clamp shall be used as a bleeder valve to regulate the vacuum to 50 to 100 mm (2 to 4 in) of Hg.

The manifold valves shall be closed and the vacuum fully opened. The applied vacuum shall be adjusted to 50 to 100 mm (2 to 4 in) of Hg using the bleeder valve on the vacuum tubing. The bleeder valve shall not be readjusted during filtration. If necessary, the vacuum shall be turned on and off during filtration at the vacuum source.

The manifold and wells shall be cleaned with hot water and detergent between each set of samples.

A.5.3 Membrane filter preparation

The filtration manifold vacuum source shall be turned on. While all the manifold well support valves are closed, one filter shall be placed on each manifold support screen. One filter position shall be used for each sample volume to be assayed, including a minimum of one positive control and one negative control each time the manifold is used. The filter wells shall be positioned firmly over each filter.

A.5.4 Sample size

A.5.4.1 The size of the sample shall be governed by expected microsphere density. An ideal sample volume shall yield 10 to 200 microspheres and not more than 500 microspheres on a membrane filter surface. The samples shall be analyzed by filtering the appropriate volume depending on the expected microsphere density. Table B.1 of this Annex contains suggested sample volumes.

A.5.4.2 When less than 10 mL of sample is filtered, 10 mL of DI water shall be added to the funnel before filtration to aid in dispersion of the microspheres over the entire filtering surface. If a pipette is used for transferring, it shall be rinsed 5 times with 0.01% polyoxyethylene sorbitan mono-oleate solution to ensure transfer of all microspheres.

A.5.4.3 When 1 L or more of sample is filtered, 1 L of sample shall be poured into a separation funnel and gradually added to the filtration manifold. When filtering larger volumes, the sample bottle shall be weighed before and after filtration to determine the volume filtered. The sample bottle and separation funnel shall be rinsed five times with 0.01% polyoxyethylene sorbitan mono-oleate solution to ensure transfer of all microspheres.

A.5.5 Sample application

1) The sample shall be well mixed and added to the manifold well.

2) Test rig blank samples shall be collected prior to the introduction of microspheres. These samples shall be analyzed if microspheres are detected in the eighth cycle effluent test samples.

3) A effluent matrix spike sample containing 50 to 100 microspheres shall also be analyzed for each test run following the procedure specified in A.6.4.

4) 1.0 mL PBS working solution shall be added to a well for a negative control (blank).

A.5.6 Filter mounting

1) The membrane filter shall be removed with a clean forceps and be applied to a 75 mm x 50 mm glass slide.

2) The membrane shall be affixed to the slide using clear nail polish. The sample number and the volume filtered shall be affixed to the slide.

3) The membrane shall air dry in a covered container.

4) The slides shall be examined microscopically within 5 d of preparation using an epifluorescence microscope equipped with appropriate filters for FTIC dye.

$$\text{number of microspheres} \setminus L = \frac{\text{count}}{\text{volume filtered (L)}} X \frac{\text{total sample volume (L)}}{\text{total sample volume (L)} - 0.001}$$

A.5.7 Computing and reporting counts

1) The EPA-ICR method 814-B-95-003,[6] Chapter 6, shall be consulted to determine the microspheres counts on membrane filters. The filter shall be scanned at 20x magnification from left to right, top to bottom, with the aid of stage scale values to eliminate any confusion between rows. If necessary, the magnification shall be increased to 40x to verify the character of the microspheres.

2) The entire filter shall be scanned. The count shall be multiplied by the appropriate factor to determine the total count per liter of sample. The following calculation shall be used to determine microsphere concentration:

3) The 99.95% reduction endpoint shall be calculated by multiplying the individual influent sample point concentration (microspheres/L) by 0.0005.

4) If the enumeration of the effluent sample is less than the 99.95% reduction endpoint but greater than (99.95% reduction – MDL), as determined in A.6.3.1, evaluation of the duplicate effluent sample shall be performed.

For example, where:

– influent concentration is 50,000 microspheres/L; and
– MDL is 12 microspheres/L.

To calculate the 99.95% reduction endpoint (step 3):

(50,000) x (0.0005) = 25 microspheres/L.

To calculate the whether the samples must be duplicated (step 4):

25 – 12 = 13.

Therefore for any effluent sample in the range of 13 to 24 microspheres/L, the sample shall be analyzed in duplicate.

A.6 Quality control

A.6.1 Minimum requirements

Each laboratory that uses this method is required to operate a formal quality assurance (QA) program. The minimum requirements of this program shall consist of an initial demonstration of laboratory capability, analysis of spiked samples to evaluate and document data quality, and analysis of blanks as tests of continued performance. Laboratory performance shall be compared to established performance criteria to determine whether the results of analyses meet the performance characteristics of the method (see Table A.2).

A.6.1.1 A test of the microscope used for detection of microspheres shall be performed prior to examination of slides. This test is referenced in EPA-ICR method 814-B-95-003.[6]

A.6.1.2 In recognition of advances that are occurring in analytical technology, certain options shall be permitted to improve detection or lower the costs of measurements provided that all quality control acceptance criteria are met. If an analytical technique other than the techniques specified in this method is used, that technique shall have a specificity equal to or better than the specificity of the techniques in this method for microspheres in the sample of interest. Specificity shall be defined as producing results equivalent to the results produced by this method for microspheres in drinking water and that meet all of the quality control (QC) acceptance criteria stated in this method.

A.6.1.2.1 Each time a modification is made to this method, the analyst shall repeat the initial demonstration of laboratory capability test in B.6.3 to demonstrate that the modification produces results equivalent to or superior to results produced by this method.

A.6.1.2.2 The laboratory shall maintain records of modifications made to this method.

A.6.1.3 The laboratory shall, on an ongoing basis, demonstrate through analysis of the effluent matrix spike sample that the analysis system is in control.

A.6.1.4 The laboratory shall maintain records to define the quality of data that is generated.

A.6.2 Micropipette calibration

A.6.2.1 Micropipettes shall be sent to the manufacturer for calibration annually. Alternatively, a qualified independent technician specializing in micropipette calibration shall be used. Documentation on the precision of the recalibrated micropipette shall be obtained from the manufacturer or technician.

A.6.2.2 Internal and external calibration records shall be kept on file in the laboratory's QA logbook.

A.6.2.3 If a micropipette calibration problem is suspected, the laboratory shall tare an empty weighing boat on the analytical balance and pipette the following volumes of reagent water into the weigh boat using the pipette in question: 100% of the maximum dispensing capacity of the micropipette, 50% of the capacity, and 10% of the capacity. If the weight of the water records within 1% of the desired weight (mL), the pipette shall be acceptable for use.

A.6.2.4 If the weight of the reagent water is outside the acceptable limits, the manufacturer's instruction manual troubleshooting section shall be consulted, and the steps described in B.6.2.3 shall be repeated. If problems with the pipette persist, the laboratory shall send the pipette to the manufacturer for recalibration.

A.6.3 Initial demonstration of laboratory capability

A.6.3.1 Method detection limit (MDL)

To establish the ability to detect microspheres, the laboratory shall determine the MDL in reagent water per the procedure in 40 *CFR* 136,[6] appendix B, using the apparatus, reagent, and standard that will be used in the practice of this method.

A.6.3.2 Initial precision and recovery

To establish the ability to demonstrate control over the analysis system and to generate acceptable precision and accuracy, the laboratory shall perform the following operations:

1) Using results of the MDL analyses, compute the average percent recovery (X) for microspheres.

2) Compare the MDL and X with the corresponding limits for precision and recovery in Table B.2. If the MDL and X meet the acceptance criteria, system performance is acceptable and the analysis of blanks and samples are able to begin. However, if any individual X falls outside the range for recovery, or if the MDL exceeds the precision limit, system performance is unacceptable for microspheres. In this event, correct the problem and repeat the test (see A.6.3.1).

A.6.4 Matrix spike

The laboratory shall spike and analyze a separate sample aliquot to determine the effect of the matrix on the method's recovery efficiency. A duplicate effluent sample shall be spiked with the appropriate volume of the enumeration microsphere stock solution as specified in A.4 to obtain 50 to 100 microspheres/L. The matrix spike shall be analyzed as described in A.5

A.6.4.1 Compute the percent recovery (R) of the microspheres using the following equation:

$$R = 100 \times (N_{sp} - N_s)/T$$

where:

R is the percent recovery;
N_{sp} is the number of microspheres detected in the spiked sample (microspheres/L);
N_s is the number of microspheres detected in the unspiked sample (microspheres/L); and
T is the spike concentration of the microspheres (microspheres/L).

A.6.4.2 The microsphere recovery shall be compared with the corresponding limits in Table B.2 until twenty recovery analyses are available, at which time the laboratory shall establish its own control limits. If the recovery for microspheres falls outside its limit, method performance for that sample is unacceptable. Corrective action shall be taken, and duplicate effluent samples shall be analyzed.

When 20 internal performance recovery data are available, control limits shall be developed from the mean percent recovery (x) and standard deviation (s) of the percent recovery. These data shall be used to generate upper and lower control limits:

– upper control limit = $x + 3s$; and
– lower control limit = $x - 3s$.

These control limits shall not exceed those in Table B.2. After every ten data points, new control limits shall be generated using the most recent twenty data points. If the recovery fall outside the control limits, method performance for that sample is unacceptable. Corrective action shall be taken, and duplicate effluent samples and an additional matrix spike sample shall be analyzed.

A.6.5 Blank (negative control sample)

If any microspheres are found in the blank, analysis of additional samples shall be halted until the source of contamination is eliminated and a blank shows no evidence of contamination. Any sample in a batch associated with a contaminated blank that shows the presence of one or more microspheres shall be

assumed to be contaminated and shall be recollected. Any sample in which microspheres are not detected shall be assumed to be uncontaminated.

A.6.6 Ongoing precision and recovery

The recovery shall be compared with the limits for recovery in Table B.2 until laboratory control limits are established as specified in B.6.4.2. If the recovery meets the acceptance criteria, system performance shall be considered acceptable. If, however, the recovery falls outside the range given, system performance shall be considered unacceptable. In this event, a problem with the microscope or with the filtration systems shall be investigated. Corrective action shall be taken, and duplicate effluent samples and an additional matrix spike sample shall be analyzed.

A minimum of one matrix spike sample shall be analyzed and shall meet the recovery criteria in Table A.2 for each performance test.

A.7 Analyst verification

A.7.1 At least once in each month during which microscopic examinations are to be performed, the principal analyst/supervisor shall prepare a slide containing 40 to 100 microspheres. The total number of microspheres determined by each analyst shall be within 10% of the number determined by the principal analyst/supervisor. If the number is not within this range, the principal analyst/supervisor and the analyst shall resolve how to identify and enumerate microspheres, and the principal analyst/supervisor shall prepare a new slide and the test shall be repeated.

B.7.2 The laboratory shall document the date, name of principal analyst/supervisor, name(s) of analyst(s), number of total microspheres placed on the slide, number determined by the principal analyst/supervisor, number determined by the analyst(s), whether the test was passed/failed for each analyst, and the number of attempts prior to passage.

B.7.3 Only after an analyst has passed the criteria in A.7.1 shall microspheres in blanks, standards, and samples be identified and enumerated.

Table A1 – Suggested sample volumes for 25 mm membrane filters

Expected sample density	Volume (x) to be filtered (mL)				
	0.1	1	10	100	1000
influent (10^5 – 10^6/L)	x	x			
influent (10^3 – 10^4/L)			x	x	
effluent (10^2 – 10^3/L)				x	x
effluent (< 100/L)					x

Table A2 – Quality control acceptance criteria for performance tests for microspheres

Performance test	Acceptance criteria
precision (as MDL)	≤ 20 microspheres/L
recovery (percent)	50 – 100

Annex B[4]

(informative)

Quality Assurance Project Plan

B.1 Introduction

The Quality Assurance Project Plan (QAPP) for this test specifies procedures that should be used to ensure data quality and integrity. Careful adherence to these procedures should 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.

B.2 Quality Assurance Responsibilities

It is possible that a number of individuals are 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.

B.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.

B.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.

B.3.2 Quality Control (QC) Checks

This section describes the recommended QC requirements for testing in this standard. The QC checks provide a means of measuring the quality of data produced.

B.3.2.1 Water QC Data

The Quality Assurance Project Plan should emphasize the methods to be employed for sampling and analytical quality assurance (QA). The important aspects of sampling and analytical QA/QC are as follows:

[4] The information contained in this annex is not part of this American National Standard (ANS) and has not been processed in accordance with ANSI's requirements for an ANS. Therefore, this annex may contain material that has not been subjected to public review or a consensus process. In addition, it does not contain requirements necessary for conformance to the Standard.

B.3.2.1.1 Chemical Analyses:

Duplicate Samples: Duplicate samples must be analyzed to determine the precision of analysis. The pertinent section of the standard should determine samples to be analyzed in duplicate. Duplicate samples measure the precision of the sampling and analysis procedures. Chemical analytical duplicates measure the precision associated only with the laboratory procedures.

*Method Blanks***:** Method blanks are used to evaluate analytical chemical method-induced contamination, which may cause false positive results.

Spiked Samples: One in ten samples is spiked with a known quantity of the chemical to be analyzed in a sample. This is a measure of accuracy of the analytical procedure.

Travel Blanks. Travel blanks should be provided to the analytical laboratory to evaluate travel-related contamination. The samples typically accompany the other samples throughout the sampling process.

Performance Evaluation Samples: Performance evaluation samples are samples of unknown concentration prepared by an independent performance evaluation (PE) lab and provided as unknowns to an analyst to evaluate his or her analytical performance. The control limits for the PE samples should be used to evaluate the analytical laboratory's method performance.

A PE sample comes with statistics that have been derived from the analysis of the sample by a number of laboratories using EPA approved methods. These statistics include a true value of the PE sample, a mean of the laboratory results obtained from the analysis of the PE sample, and an acceptance range for sample values. The analytical laboratory is expected to provide results from the analysis of the PE samples that meet the performance capabilities of the verification testing.

B.3.2.1.2 Microbiological Analyses:

The following are recommended quality-control samples for testing and reflect sound laboratory practices.

Equipment blanks – collect influent and effluent water samples without the addition of challenge microorganism passing through the equipment. The blanks should be collected at least once on each day of testing and the concentration of challenge microorganisms should be negligible such as less than 0.5 $\log_{10}$.

Negative control - The negative control is an experiment done with a sample/mock sample that should normally yield a negative result. It allows to check for contamination of the reagents or artifacts that would give false positive.

Trip controls – collect one sample of the challenge microorganism stock solution that travels with the stock solution used for testing from the microbiological laboratory to the location of equipment tested and back to the laboratory. The change in the log concentration of the challenge microorganism in the trip control should be within the measurement error. The measurement error should be on the order of 3 to 5 percent or +/- 0.25 $\log_{10}$.

M*ethod blanks (MB)* – An MB is a sample bottle of sterilized reagent grade water that undergoes the challenge microorganism assay procedure. The concentration of challenge microorganism with the method blank should be non-detectable, according to *Standard Methods for the Examination of Water and Wastewater* (APHA et al. 1998).

Stability samples – samples of influent and effluent are collected to assess the viability of the challenge microorganism concentration over the time period from sample collection to completion of challenge microorganism assay. The challenge microorganism concentrations in the stability samples should be within 5 percent of each other or +/- 0.25 $\log_{10}$.

Replicate analyses (plating): Each sample should be plated in triplicate and the average microbial value for the sample calculated from the three plate replicates. The typical way to calculate the average of a microbial sample is to calculate the geometric mean of the three replicates (triplicates).

B.3.2.2 Instrumentation QC:

During testing, all equipment used in testing should be calibrated to minimize uncertainty. For example, all measurements of flow rate, pressure, temperature and other instrument measurements that could affect performance should be calibrated with controls traceable to an independent standard such as NIST or equivalent with a known measurement uncertainty.

B.3.3 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:

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

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.

B.3.4 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.

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

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%.

B.3.5 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.

B.3.6 Completeness

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

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

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

Completeness is defined as follows for all measurements:

$$\%C = (V/T) \times 100$$

where:

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

Retesting should 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.

B.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.

B.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 should be flagged, or not reported.

B.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.

B.5 Testing Inspections

The NSF 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.

Annex C[5]

(informative)

Data Management, Analysis, and Reporting

C.1 Data Management and Analysis

All operational and analytical data should be gathered and included in test report. The data should consist of results of analyses and measurements and QA/QC reports.

C.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.

C.3 Performance Reporting

C.3.1 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.

C.3.2 Information on Liquid Contact Angle and others for Annex C Data Management, Analysis, and Reporting.

Liquid-membrane contact (i.e., “wetting”) angle is measured in degrees and indicated by Θ. The Θ value is used in equations to achieve a resolution of 3 µ with pressure-based direct integrity tests. The pressure applied during the test must be great enough to overcome the capillary forces in a 3 µ hole thus ensuring that any breach large enough to pass *Cryptosporidium* oocysts would also pass air during the test. The amount pressure needed to achieve a 3 µ resolution is important to compliance the LT2ESWTR.

[5] The information contained in this annex is not part of this American National Standard (ANS) and has not been processed in accordance with ANSI’s requirements for an ANS. Therefore, this annex may contain material that has not been subjected to public review or a consensus process. In addition, it does not contain requirements necessary for conformance to the Standard.

The liquid-membrane contact angle ranges from 0-90^{o} and is primarily a function of the membrane hydrophilicity, which can be characterized in general terms as the affinity of the membrane material for water or the ability of the membrane to become wetted with water. For an ideally hydrophilic membrane, the liquid-membrane contact angle is 0 degrees. Although many membranes used for drinking water applications are manufactured using hydrophilic materials, an ideally hydrophilic membrane is purely theoretical.

The Θ value is unique to a membrane material and type. In the absence of data supplied by the membrane manufacturer, a conservative value of Θ = 0 is suggested in the LTESWTR MFGM. Because a less conservative contact angle can significantly reduce the minimum required integrity test pressure, any value for Θ other than 0 degrees should be well-documented and approved by the State if used for the purposes of regulatory compliance, such as under the LT2ESWTR.

Log Removal Value (LRV) estimate from PDT test data

When using PDT data, an LRV can be estimated by calculation: LRV_{calc}. The LRV_{calc} can be calculated using an equation and measurement during the PDT. However, there are some assumptions which should be discussed and resolved.

The equation for the LRV_{calc} calculation is:

$$LRV_{calc} = \log [(Qp * ALCR*Patm)/(\Delta Ptest*Vsys*VCF)]$$

The terms in the equation are:
Qp - flow measured prior to testing (average of pre and post challenge flow measured);
ALCR – air-liquid conversion ratio (dimensionless) and read discussion on ALCR;
Patm - atmospheric pressure at sea level = 14.7 psi;
Δptest - decay rate in psi/min (pre and post challenge average);
Vsys - volume (L) of pressurized air in the system during the test which is the hold-up volume;
VCF - value for deposition mode = 1

The equation for ACLR calculation is:

$$ACLR = 170 \times Y \sqrt{((Ptest-BP)* \times (Ptest+ Patm)\div[(460+T)*TMP])}$$

The terms of the equation are:
Y = net expansion factor for compressible flow through a pipe to a larger area (dimensionless) but see Crane 1988. Shall we assume isothermal flow through fibers? The range from Page A-22 of Crane are 0.588 - 0.718. So shall we use the middle of the range?
Ptest - direct integrity test pressure (psi);
BP - backpressure on the system during the integrity test (psi) which is always 0 as it is open to the atmosphere;
Patm - atmospheric pressure (psia) and the atmospheric pressure at sea level = 14.7;
T - water temperature (F);
TMP- trans-membrane pressure during normal operation (psi) which is difference between inlet and outlet measured pressure during testing.
Are the assumptions acceptable?

Information from Manufacturer:

Table X-Y. Make and Model Module Specifications	
Parameter	**Value**
Dimensions:	
Nominal Membrane Pore Size	
Fiber Inner Diameter	
Fiber Outer Diameter	
Module diameter	
Module length	
Membrane surface area	
Filtration Flow Direction	
Operating Limits:	
Maximum certified flux at 20 °C	
Maximum certified flow at 20 °C	
Operating temperature range	
Maximum feed pressure	
Maximum transmembrane pressure (TMP)	
Operating pH range	
Maximum chlorine tolerance	
Manufacturing NDPT	
Method	
Quality Control Release Value	

C.4 Report of Equipment Testing

The report should be issued in draft form for review prior to final publication. The reports should 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.

Annex D[6]

(informative)

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

[6] The information contained in this annex is not part of this American National Standard (ANS) and has not been processed in accordance with ANSI's requirements for an ANS. Therefore, this annex may contain material that has not been subjected to public review or a consensus process. In addition, it does not contain requirements necessary for conformance to the Standard.

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.

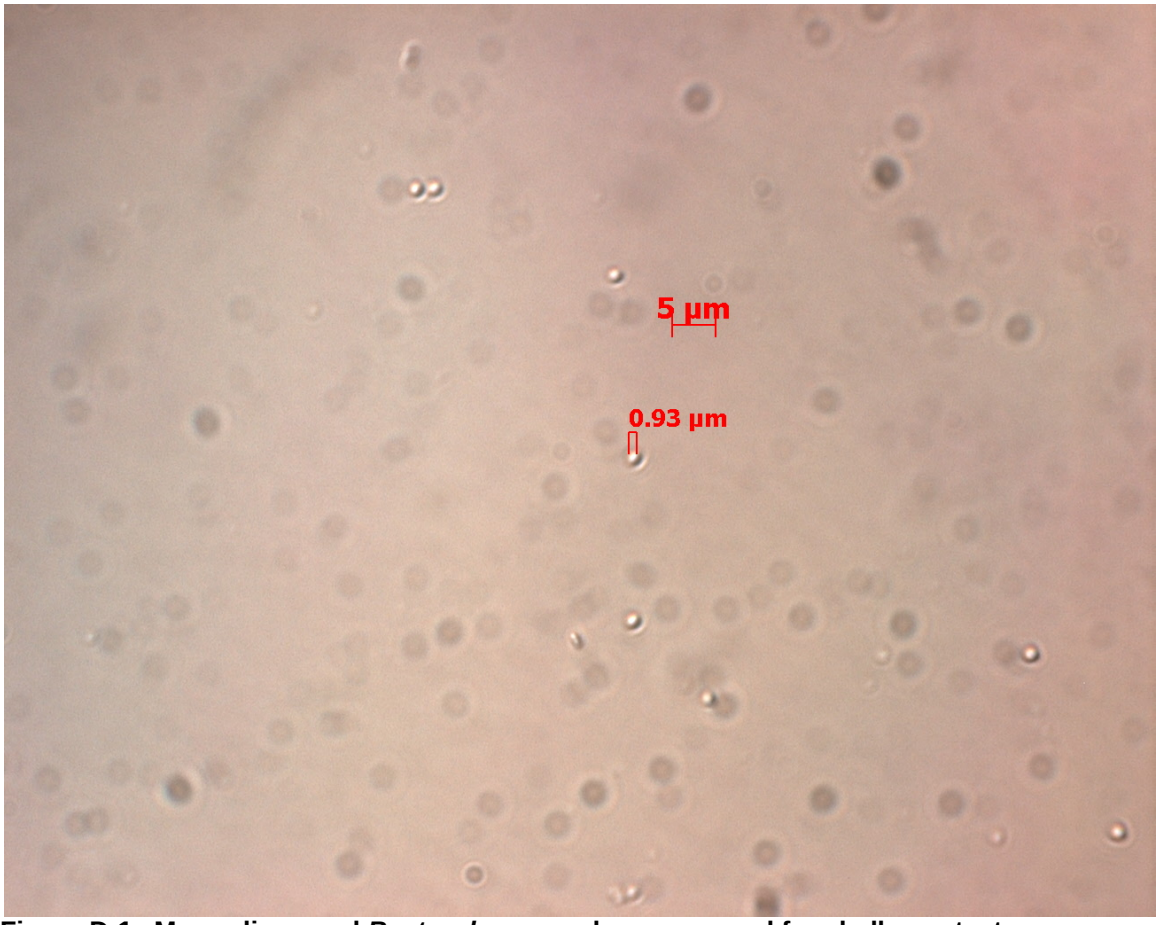

Figure D-1. Mono-dispersed *B. atrophaeus* endospores used for challenge tests.

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Annex E
(informative)

Validation testing for microspheres as surrogates for oocysts

Summary Report to the DWTU *Cryptosporidium* Task Group on the Filtration Efficiency Comparison Study

May 3, 1996

Submitted by:

Robert Herman
NSF *International*
Engineering and Research Services

1.0 Purpose of Study

The purpose of this study is to provide the DWTU *Cryptosporidium* Task Group adequate data to recommend to the DWTU Joint Committee a validated test method. The study design was developed to address requests from the Task Group. The requests were:

- Generate data using test dust loading with microsphere challenge particles.
- Generate data using test dust loading with live oocyst challenge.
- Correlate ANSI/NSF Standard 53 test dust data with microsphere and live oocyst data.
- Generate data using live oocyst challenge without the addition of test dust.

2.0 Technical Approach

The tests using oocysts or microspheres with test dust loading was performed as close to the current ANSI/NSF Standard 53 protocol as possible. Two types of DWTUs were used, a membrane filter and a depth filter. To accommodate the use of oocysts or microspheres there were several modifications to the protocol. The modifications and test parameters were:

- 50% on/50 % off cycle with a 20 minute cycle time.
- Oocyst analysis were done by an antibody staining, microscopic technique
- Fluorescent microsphere analysis were done by microscopic examination.
- At each sample point, test dust challenge was terminated and the challenge particle was introduced. Sampling occurred at the beginning of the third cycle after starting the oocyst or microsphere challenge. Once the sampling was complete, test dust loading was resumed.
- Influent oocyst or microsphere challenge concentration was adjusted to accommodate the challenge particle and analytical methodology.
- The live oocyst data was generated by not initiating the test dust loading to the above test until after a sample at the fourth and eighth cycles were taken.

Two tests were performed using the live oocysts to generate data for both the membrane and depth filters. Two tests were performed using microspheres for both filter types. Test data was collected from recent ANSI/NSF Standard 53 tests (0-5 micron dust).

3.0 Procedures

3.1 Test Units

Two units were selected from filters submitted to NSF for cyst reduction but had not maintained 99.95% reduction. Units which had failed were selected because the purpose of the study is to compare the reduction percents of three different particles so the test filters needed to pass some of the challenge particles to provide measurable values. The membrane filter unit was a pleated

nylon membrane in a double open ended 10" cartridge and the depth filter unit was a compressed non-cellulose fiber impregnated with carbon. Both units used flow-limiting orifices to control the maximum flow rate.

3.2 Challenge Particles

The test dust used in all of the tests was certified 0-5 micron dust obtained from Particle Technologies Inc. The polystyrene microspheres were obtained from Bangs Laboratories and were 2.355 micron with a 0.026 micron standard deviation. They were dyed fluorescent orange with DCM. The *Cryptosporidium parvum* oocysts were obtained live from Waterborne, Inc. and were obtained from calf and human donors.

3.3 Test Rigs

The test rig used for the 0-5 micron dust test was the California laboratory batch test rig as described in ANSI/NSF Standard 53. The test rig used for the microsphere and oocyst tests was developed specifically to allow pathogen testing and deployed direct injection of challenges. Figure 1 gives a basic description of the test rig used.

3.4 Influent Parameters

The influent water characteristics were maintained at the required levels throughout all tests. The test units were cycled at a 50% on/50% off ratio with a 20 minute cycle time.

3.5 Test Dust Loading

The test dust for the Standard protocol was set to provide the proper number of 3-4 micron particles as the influent challenge. In the microsphere and oocyst tests, the test dust was used to load the filter media and produce a flow reduction as the filter clogged. The test dust was loaded at 15 to 20 NTU. This range was selected to simulate the turbidity range seen in most cyst reduction tests.

3.6 Sampling

The test dust procedure followed the Standard protocol which had samples at the beginning of the 4th cycle, and at 25, 50, and 75 percent flow reduction. The microsphere and oocyst procedure called for an additional sample point at the beginning of the 8th cycle. The challenge particle was introduced only during the sample point. At the beginning of the test, the microsphere or oocyst particles were introduced without test dust for a full 8 cycles. At all other sample points, the test dust was stopped and the challenge particle was introduced with sampling occurring at the beginning of the 3rd cycle. On most occasions, when the test dust was stopped and the challenge particle

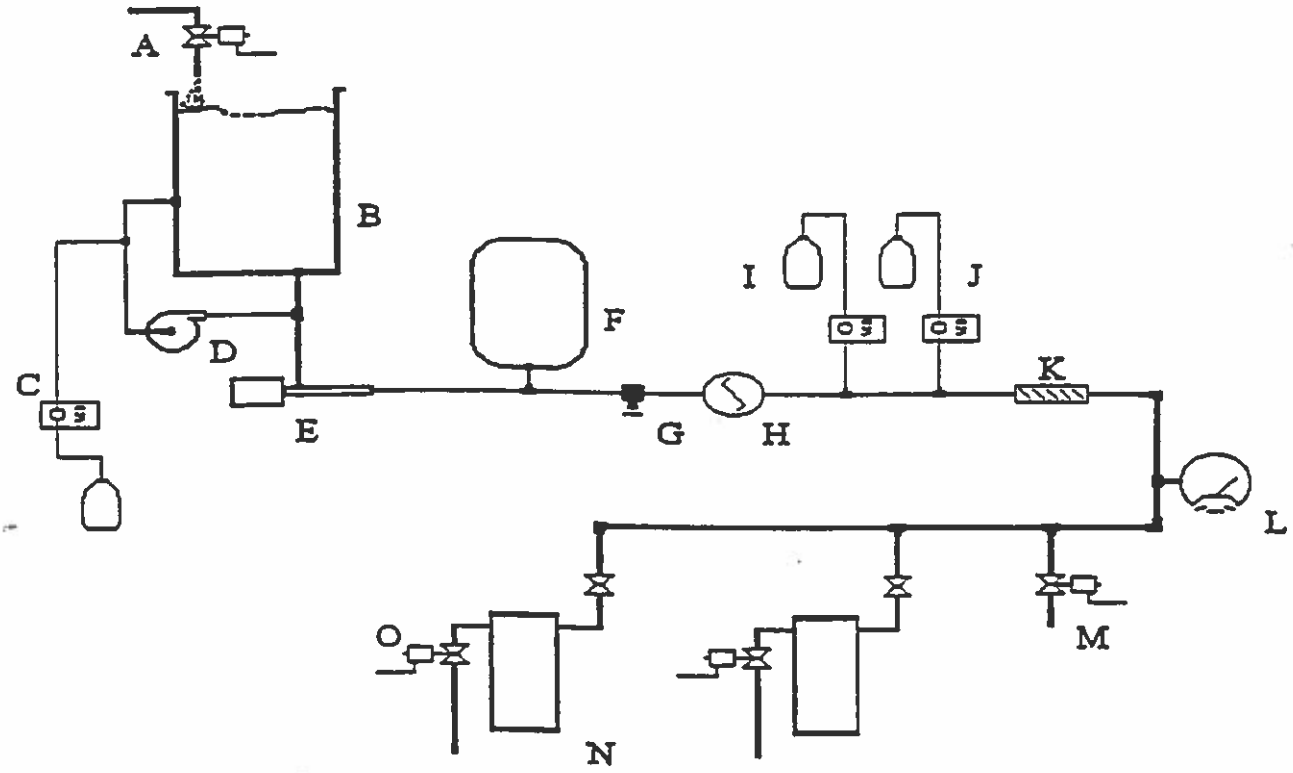

Figure 1. Direct Injection Infectious Agent DWTU Test Rig

A. City water supply
B. Mixing tank with temperature control
C. Acid injection for pH control
D. Recirculation pump
E. Booster pump
F. Bladder tank
G. Pressure regulator
H. Flow meter
I. Test dust injection
J. Microsphere or oocyst injection
K. In line mixer
L. Pressure gauge
M. Influent sample port
N. Test units
O. Cycling control solenoid valves

began, the flow reduction decreased 2 - 3 percent. This was compensated in later tests by running the test dust to 3 percent past the sample point.

3.7 Challenge Particle Analysis

The ANSI/NSF Standard 53 test dust was analyzed by particle counter as specified by the Standard. An epi-fluorescent microscope was used to count the influent and effluent concentrations of microspheres and oocysts.

The microsphere counting method involved passing a measured volume of sample through a 47mm 0.45 micron membrane filter and counting the microspheres under the microscope. Influent concentrations were determined by counting 10 random areas and factoring to determine the total count. Effluent concentrations were determined by the same method if very high counts were observed, if less than 4 microspheres were observed in each area, the entire filter was counted.

The oocyst analysis involved the filtering of a known volume of sample through a 25 mm membrane and performing a direct antibody staining technique to stain the oocysts fluorescent apple-green. Ten mL of the influent samples were passed through the filter and the entire filter was counted. If the effluent samples contained high counts the same procedure was performed as the influent samples. If the effluent samples contained low counts, a 1 liter sample was centrifuged, concentrated, and passed through the filter. In all cases, the entire filter was scanned and counted.

4.0 Test Results

The results for the microsphere test, live oocyst test, and ANSI/NSF Standard 53 test are summarized in Tables 1 and 2. Log reduction comparison plots are included after each table as figures 2 and 3.

4.1 Pleated Membrane Filter Results

The pleated membrane filter removed the oocysts at greater than 2 log reduction for the beginning of the test and dropped significantly to less than 1 log reduction at the 25% sample point through the end of the test. The steady loss of reduction efficiency was observed with the microspheres but with less emphasis. The reduction throughout the study was less than 1 log. The 75% sample point for Unit A had a significant amount test dust in the sample which interfered with the counting. The test dust obscured many of the microspheres making it difficult to positively identify them and caused a low count. The ANSI/NSF Standard 53 test dust reduction percent did drop through the study but the high variability of the results made the trend less clearly defined.

4.2 Compressed Fiber Filter Results

The compressed fiber filter removed the oocysts at greater than 4 logs at the beginning of the test and dropped to just above 2 logs at the 25 percent flow reduction sample point. The reduction recovered to 3 log reduction at the 50 and 75 percent sample points. This pattern of the lowest reduction at the 25 percent sample point was repeated for all three of the challenge particles. The ANSI/NSF Standard 53 test followed the oocyst reduction through the entire test. The 2.355 micron microspheres began at greater than 4 log reduction and dropped to 1 log reduction at the 25 percent sample point and recovered to only 1.5 log at the 50 and 75 percent sample points. The microsphere log reduction followed approximately 1.5 log lower than the oocyst reduction.

The data for compressed fiber filter unit B in the oocyst test was not plotted on the log reduction chart because of the questionable nature of the results. The poor performance of unit B and lack of reaching the 25 percent flow reduction even though 23 percent more influent passed through the filter indicated that unit B had a manufacturing defect which allowed channeling of the oocysts through the media.

5.0 Discussion

For both filter types the highest reduction percents were observed at the 4th and 8th cycle sample points. These sample points were unique to the studies since they were taken while the filter media were clean and had not been exposed to test dust. Both of the filter assemblies used flow restriction orifices to limit the flow through the units at the beginning of the test. This resulted in most of the pressure drop through the unit to be applied across the orifice and very little pressure drop across the filter media. This low pressure drop across the filter media minimized the stress on the filter media and supplied little force to flex the oocysts through the filter media.
The depth filter may have another effect which would improve the reduction at the beginning of the test. Since the depth filter functions by surface and matrix trapping of the particles, the rate of migration of the particles through the media may be slower than the sample period at the beginning of the test.

5.1 Pleated Membrane Filter

The oocysts were reduced by 2-3 logs at the beginning of the test. This would indicate that the membrane had a significant number of pores in the 4 - 5 micron range to pass oocysts without significant pressure drop across the filter media. The microspheres were reduced by less than 1 log at the beginning of the test. Approximately 25 to 30 percent of the microspheres passed through the filter media. This would give evidence that the 2.355 micron microspheres were close to the median pore size of the filter. The 3 - 4 micron test dust was reduced by greater than 4 logs at the beginning of the test. This would tend to indicate that the pore size of the filter media was much lower than 3 microns. The evidence from the oocysts and microspheres does not

Table 1. Pleated Membrane Filter Test Data Summary

Sample Point	Influent (particles)	Effluent A (particles)	Effluent B (particles)	% Reduction A	% Reduction B	Flowrate A (gpm)	Flowrate B (gpm)	% Flow Reduction A	% Flow Reduction B	Gallons Treated A	Gallons Treated B
Pleated Membrane Microsphere Test (counts in #/L)											
4th Cycle	31000	9700	9500	68.710	69.355	4.1	4.2	-	-	125	125
8th Cycle	51000	12000	11000	76.471	78.431	4.1	4.2	-	-	292	293
25% Flow Red.	40000	16000	26000	60.000	35.000	3.4	3.2	17	24	2228	2219
50% Flow Red.	98000	71000	86000	27.551	12.245	2.4	2.4	42	43	3026	2968
75% Flow Red.	96000	21000*	53000	78.125*	44.792	1.0	1.1	76	74	3664	3615
Pleated Membrane Live *Cryptosporidium parvum* Oocyst Test (counts in #/L)											
4th Cycle	13000	72	69	99.446	99.469	3.4	3.3	-	-	95	101
8th Cycle	17000	23	13	99.865	99.924	3.4	3.3	-	-	198	203
25% Flow Red.	23000	2055	1500	91.065	93.478	2.5	2.7	26	18	3141	3148
50% Flow Red.	30000	2600	1600	91.333	94.667	1.6	1.7	53	48	4150	4296
75% Flow Red.	20000	3500	7600	82.500	62.000	0.7	0.8	79	76	4883	5036
Pleated Membrane ANSI/NSF Standard 53 Test Dust (counts in #/mL)											
4th Cycle	48000	0	0	>99.998	>99.998	4.5	4.5	-	-	40	37
25% Flow Red.	61000	13	2	99.979	99.997	3.0	3.3	33	27	650	760
50% Flow Red.	550000	37	1	99.993	>99.999	2.2	2.0	51	56	1290	1320
75% Flow Red.	230000	130	5	99.943	99.998	1.1	1.3	76	71	2040	2060
Pleated Membrane ANSI/NSF Standard 53 Test Dust Partial Data (counts in #/mL)											
4th Cycle	48000	0	1	>99.998	99.998	4.5	4.3	-	-	26	23
25% Flow Red.	77000	11	2100	99.986	97.273	3.7	3.5	18	19	760	620
50% Flow Red.	59000	57	100	99.903	99.830	-	-	-	-	2090	1780

*Test dust in the effluent obscured some of the microspheres and caused a low count.

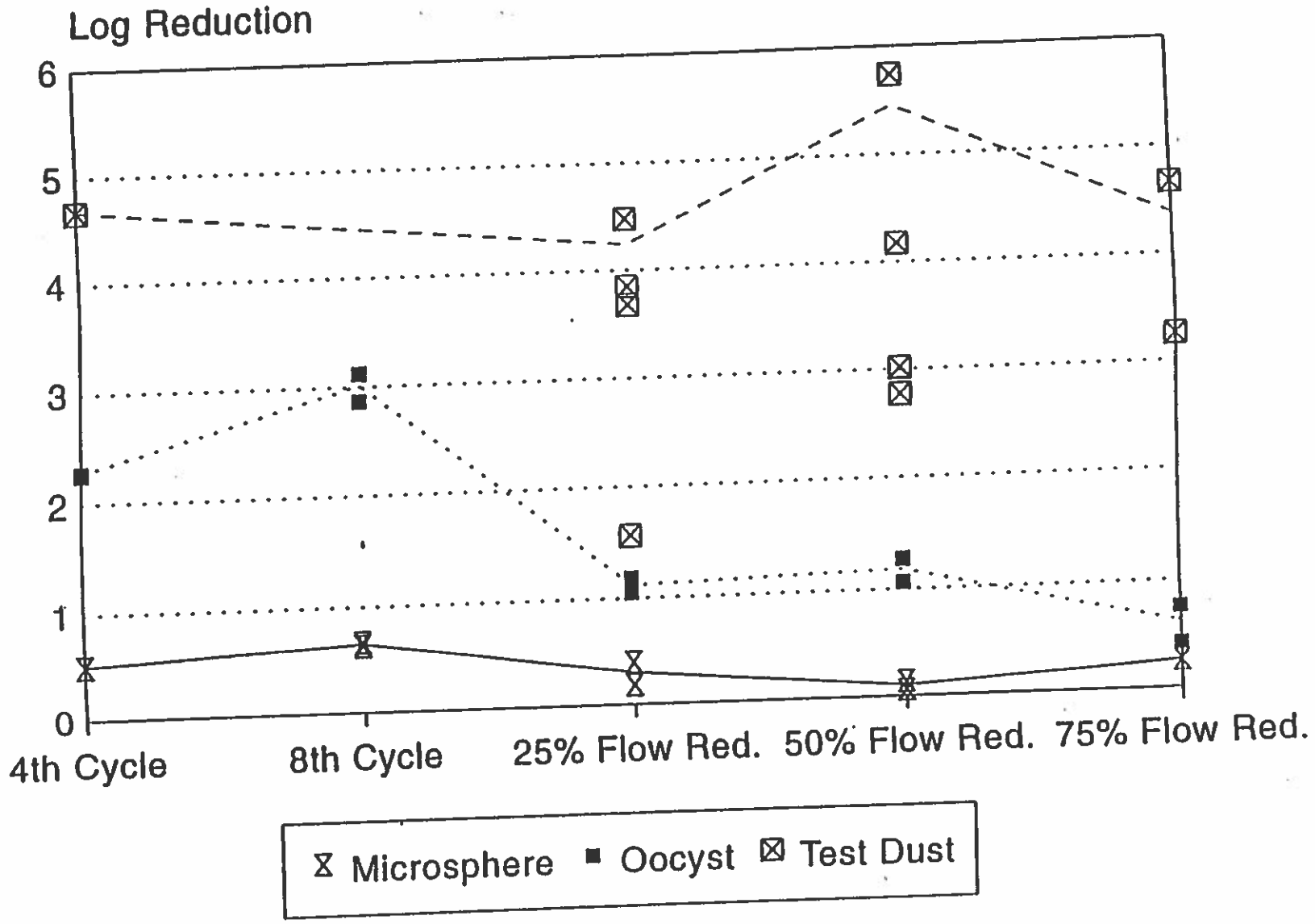

Figure 2. Pleated Membrane Filter

Table 2. Compressed Fiber Filter Test Data Summary

Sample Point	Influent (particles)	Effluent A (particles)	Effluent B (particles)	% Reduction A	% Reduction B	Flowrate A (gpm)	Flowrate B (gpm)	% Flow Reduction A	% Flow Reduction B	Gallons Treated A	Gallons Treated B
Compressed Fiber Microsphere Test (counts in #/L)											
4th Cycle	70000	2	2	99.997	99.997	0.52	0.58	-	-	16	17
8th Cycle	110000	140	352	99.873	99.680	0.52	0.58	-	-	32	33
25% Flow Red.	95000	20000	5000	78.947	94.737	0.41	0.45	21	22	440	486
50% Flow Red.	61000	4000	1000	93.443	98.361	0.27	0.28	48	52	494	537
75% Flow Red.	56000	2100	3000	96.250	94.643	0.14	0.14	73	76	576	610
Compressed Fiber Live *Cryptosporidium parvum* Oocyst Test (counts in #/L)											
4th Cycle	170000	5	81	99.997	99.952	0.59	0.70	-	-	18	22
8th Cycle	180000	9	81	99.995	99.955	0.59	0.70	-	-	37	43
25% Flow Red.	120000	904	23700	99.247	80.250	0.43	0.62	27	11	396	487
50% Flow Red.	180000	94	2700	99.948	98.500	0.26	0.36	56	49	473	607
75% Flow Red.	60000	32	2000	99.947	96.667	0.14	0.16	76	77	546	689
Compressed Fiber ANSI/NSF Standard 53 Test Dust (counts in #/mL)											
4th Cycle	130000	1	2	99.999	99.998	0.76	0.73	-	-	6	6
25% Flow Red.	71000	650	160	99.085	99.775	0.50	0.50	34	31	650	650
50% Flow Red.	48000	23	30	99.968	99.958	0.38	0.37	50	49	1100	1100
75% Flow Red.	51000	67	21	99.906	99.970	0.30	0.30	60	59	1200	1200
Compressed Fiber ANSI/NSF Standard 53 Test Dust Partial Data (counts in #/mL)											
4th Cycle	270000	14	6	99.995	99.998	0.71	.071	-	-	19	13
25% Flow Red.	310000	35000	19000	88.710	93.871	0.51	0.53	28	25	452	477

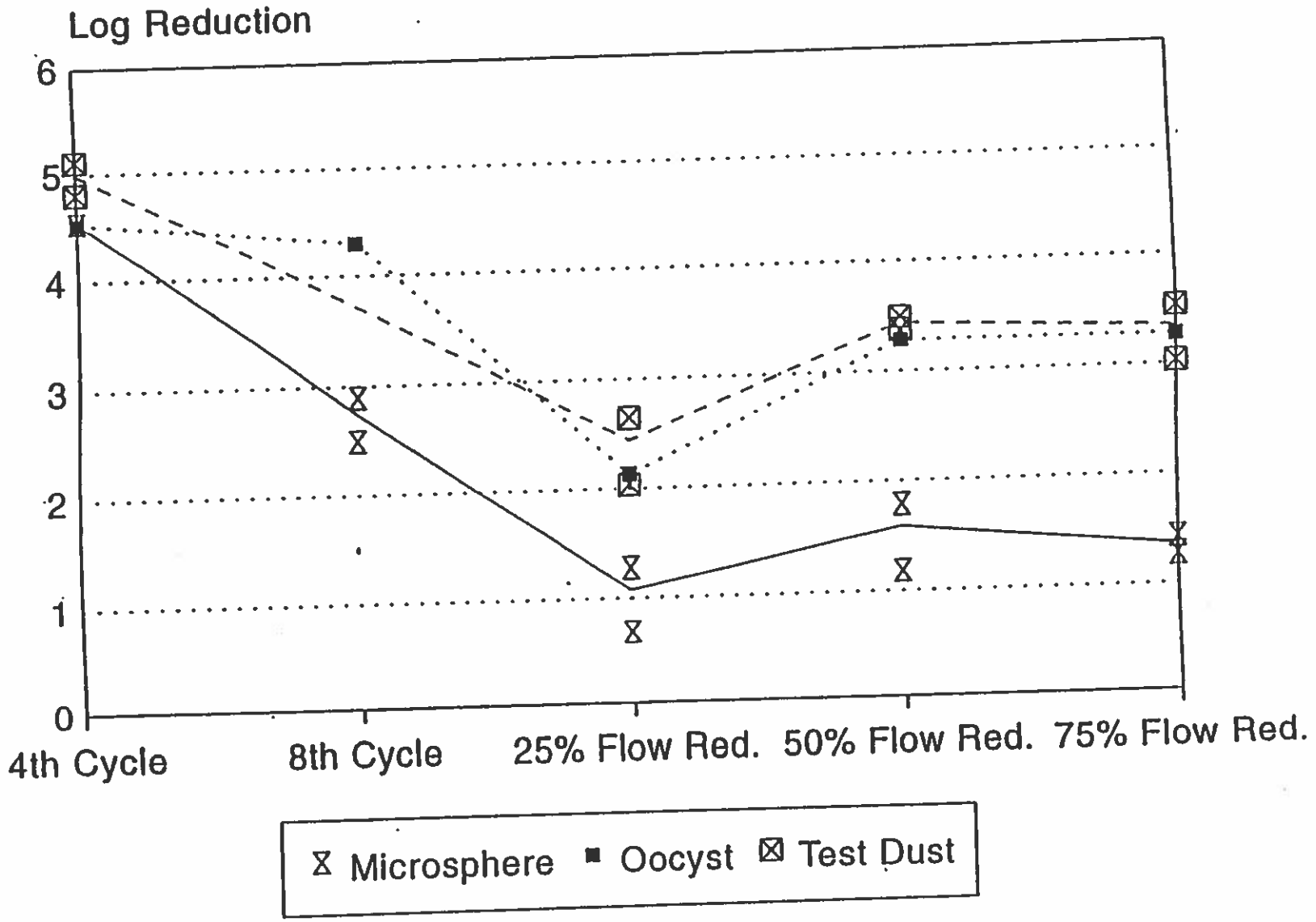

Figure 3. Compressed Fiber Filter

substantiate this conclusion. A possible cause for this inconsistency could be the nature of the test dust. The random shaping and charge of the test dust could promote bridging and adhesion to the filter media.

As the pressure drop across the membrane increased, the reduction of the oocysts and microspheres decreased significantly. The reduction of the microspheres began at approximately 70 percent at the start of the test and dropped to less than 30 percent at the 50% sample point. This change in the reduction percent could be related to the effect of the pressure drop across the filter media. Since the microspheres are inflexible, this increase is primarily due to the deformation of the filter media which allowed the microspheres to pass through. The ANSI/NSF Standard 53 test dust also performed in a similar manner as the microspheres. The reduction percent lessened as the pressure drop across the filter media increased. The oocyst change in reduction percent is more dramatic. The reduction began at 2.3 logs and dropped to less than 0.6 log (72 percent) at the 75% sample point. This near 100 fold increase in oocysts passing through the filter media indicates the ability of the oocysts to alter their shape to pass through smaller pores. If the 3 fold change in the microsphere reduction is indicative of the media deformation then the majority of the change seen in the oocyst reduction must be due to the flexibility of the live oocysts.

The microspheres were a conservative indicator of oocyst reduction for this pleated membrane filter. The oocyst reduction dropped to only 72 percent at the last sample point. This indicates that for this type of membrane filter the oocysts were able to deform enough to penetrate the membrane at nearly the same rate as rigid 2.355 micron spheres.

The ANSI/NSF Standard 53 test dust data had significantly greater reduction percents than the oocysts throughout the study. This would indicate that for this pleated membrane filter the measurement of 3 - 4 micron test dust is not a conservative indicator of oocyst removal performance.

5.2 Compressed Fiber Filter

The compressed fiber filter had the highest reduction at the 4th cycle sample point. All three particle types were at over 4 log reduction. This would most likely be due to the rate of passage of the oocysts, microspheres, and test dust through the filter media. At the 4th cycle sample point the bulk of the particles did not have adequate time to migrate through the filter media. The reduction percent lowered at the 8th cycle sample point which would indicate that adequate time had elapsed to allow the particles to migrate through the filter media.

The point of lowest particle reduction was consistently at the 25% sample point. The cause for this effect is most likely specific to this filter media and may involve the compressibility of the media. The 25% sample point is the first sample point where there is a significant pressure drop across the filter media but the pressure drop is not as high as the 50 and 75% sample points. It is possible that the media is compressed at the later sample points which increases the reduction efficiency. This would account for the increase in reduction percents at the 50 and 75% sample points.

Once the initial migration of the particles through the filter media is complete (the 8th sample point), the reduction percent comparisons can be made between the oocysts and microspheres. The microsphere reduction followed the same pattern as the oocysts but with an average 1.5 log lower particle reduction. This would indicate that for this compressed fiber filter media, the 2.355 micron microspheres are a very conservative estimator of oocyst reduction.

The ANSI/NSF Standard 53 test does not generate an 8th sample point but from the 25 percent sample point through the end of the test, the reduction of the oocysts and test dust is extremely close. Statistically there is no significant difference between the two sets of data. The partial test dust data presented in Table 2 is substantially lower than the oocyst reduction. This provides evidence that the test dust is a good indicator for oocyst reduction for this filter media.

5.3 Sources of Error

The most significant source of error in this study is the variation between filter cartridge samples. The compressed fiber filter used in the oocyst study as unit B had a manufacturing defect which required the data for that filter unit to be disregarded. Variation between duplicates and between different tests has not been addressed in this testing and could adversely effect the results. Cost limitations did not allow an adequate number of replicates to determine the confidence interval of the results.

Each particle type required a different analytical process. This allows differences in accuracy to skew the results. The oocyst and microsphere analyses used an epi-fluorescent microscope with filtration of the sample and the test dust analysis used a particle counter.

The oocyst analysis requires several steps and each step could cause a significant loss of sample. Centrifugation resulted in a loss of approximately 25% and a difference of 20% was seen between indirect and direct antibody staining techniques. These effects are most likely not linear with sample concentration so oocyst results would tend toward higher reduction percents from true values.

Microspheres required much less handling than oocysts but test dust in the effluent caused the poor results on the unit A 75% sample for the pleated membrane filter. The use of the area averaging technique allows for significant error if the particles are not evenly distributed over the surface of the filter. Agglomeration of the microspheres also makes counting difficult.

Test dust is analyzed by a particle counter which cannot identify the source of the particle and determine if the particle is a challenge particle or a carbon fine or agglomeration. Significant losses can also be attributed to adsorption onto glassware or sample bottles. With the use of test dust the sizing and channel calibration of the particle counter is critical. Small errors in channel sizing calibration can have a large effect on the influent counts.

The ANSI/NSF Standard 53 protocol requires the test dust to be in the challenge water at the proper concentration at all times. The modified protocol for the oocyst and microsphere tests provided the challenge particles only during the first 8 cycles and the 2 cycles prior to each sample

point. This may have had an effect on the reduction percents observed for the compressed fiber filter. If the migration of the particles through the device is a slow process, then the length of time the challenge particles are fed to the filter before the sample point is critical to obtain proper reduction percents.

Filtration Efficiency Comparison Study II

by

Lisa Quiggle, Project Manager, Standards

Executive Summary

This study was done to validate a testing protocol for the removal of *Cryptosporidium parvum* oocysts from drinking water by Drinking Water Treatment Units. The current ANSI/NSF Standard 53, Drinking Water Treatment Units - Health Effects cyst reduction protocol was modified to permit the use of live oocysts and latex microspheres on the same filter unit. This enabled the comparison of the oocyst and microsphere reduction without the confounding factor of filter manufacturing processes. This study also acquired data on the microbiological counting methods and the precision of these methods used in evaluating pass/fail criteria.

The study was preformed on two filters, a carbon block and a precoat filter, which had passed the current cyst reduction protocol. The study found that these filters passed the 99.95% reduction of the challenge influent for both the oocysts and the microspheres. The oocysts were not detected in the filter effluent for these two filters while a small number of microspheres were detected. The number of microspheres that were detected were below the pass/fail criteria which means that the microspheres will provide a conservative challenge. The 95% confidence interval for the oocysts counts on the influent values were 16.1%. The 95% confidence interval for the effluent values were 30.5%.

It is recommended that the live Cryptosporidium oocyst testing protocol be added to Standard 53 with the provisions of testing two filters to test manufacturing differences and establishing the acceptance criteria to 'no oocysts detected' in the effluent. This acceptance criteria would provide a safety factor considering the confidence intervals obtained by the counting methods. The criteria of 'no oocysts detected' has also been discussed by regulatory agencies as the proposed criteria for drinking water.

It is also recommended to use the 2.967 micron microspheres as a surrogate for the oocysts. This surrogate could be used on large filters that cannot be isolated due to their size and would provide a cost effective alternative to live oocysts. This surrogate could be used on large bottle water plant filters which have very high flow rates which may be prohibitively expensive to test with live oocysts.

I. Purpose of Study

This study was initiated to continue the *Cryptosporidium* Filtration Efficiency Study (Appendix A), reported to the Drinking Water Treatment Unit (DWTU) Joint Committee on April 30, 1996.

The initial study tested two filters, a pleated membrane filter and a non-cellulose fiber filter impregnated with carbon, for filtration efficiency. These filters had not maintained a 99.95% reduction of Standard 53 test dust in previous testing. The filters were tested with three challenges; *Cryptosporidium parvum* oocysts, microspheres and Standard 53 test dust. In the first study the filters were tested in duplicate using a different set of filters for each challenge reduction. It was found that a significant source of error was introduced by performing each challenge reduction on a separate filter. The initial study used polystyrene microspheres of 2.355 micron in diameter as a surrogate for the oocysts. This size of microsphere was shown to break through the filters earlier and at a significantly higher level than the *Cryptosporidium* or the Standard 53 test dust.

The Drinking Water Treatment Unit *Cryptosporidium* Task Group reviewed the initial study and requested that the study be continued to acquire additional data and to address the sources of error identified in the initial study. The following objectives were set for this study to accomplish these goals:

- Generate data using depth and 'membrane' technology DWTUs that have passed ANSI/NSF Standard 53 cyst reduction.
- Generate data using test dust, live oocysts and microspheres from the same test unit to minimize performance differences due to unit manufacturing variations.
- Correlate test dust data, microsphere data and live oocyst data.
- Validate microscopic test methods.
- Compare the results between manual microscopic counting methods and automatic counting.
- Investigate the use of on-line particle counting.
- Evaluate the use of microspheres in the 3 micron size range.

II. Technical Approach

The tests using oocysts and microspheres with test dust loading were performed as close to the current ANSI/NSF Standard 53 protocol as possible. Two types of DWTU filters were used, a precoat filter and a carbon block depth filter that had passed the Standard 53 test dust reduction. A third DWTU filter was run to gather information on the media migration phenomenon and to determine possible interferences in each test method. The Standard 53 protocol was modified to perform the oocyst, test dust and microsphere reduction on the same filter. The modifications to the protocol and test parameters are as follows:

- 50/50 cycle with a 20 minute cycle, 10 minutes on Filter A and 10 minutes on Filter B.
- Oocyst analysis was done by an antibody staining microscopic technique.
- Fluorescent microsphere analysis was done by microscopic examination.
- Particle counting was performed by taking grab samples in the same manner as the current ANSI/NSF Standard 53 test is conducted.
- At each sample point, test dust challenge was terminated and the units operated for four full cycles and then the oocyst and microsphere particles were introduced. Grab samples were taken at the beginning of the fourth cycle after starting the oocyst and microsphere challenge. Once the sampling was complete, test dust loading was resumed. Test dust grab samples were taken before the test dust challenge was terminated.
- Influent oocyst or microsphere challenge concentrations were adjusted to accommodate the challenge particle and analytical methodology at concentrations adequate to establish a minimum 4 log reduction. Test dust concentrations were maintained at $5 \pm 3 \times 10^4$ particles/ml in the 3 - 4 micron size range.
- The live oocyst and microsphere data were generated by not initiating the test dust loading to the test units until after a sample at the fourth and eighth cycles were taken.
- Microspheres with a 2.967 micron mean diameter and a 0.037 micron standard deviation were used. The microspheres were identified with a fluorescent dye that can be distinguished from the oocyst fluorescent dye.
- An on-line particle counter was used to monitor the influent and effluent throughout the study.
- Validation of the microscope methods of analysis were performed by Simin Abrishami with review to be performed by Dr. Joan Rose.

A. Test Units

Two units were selected from filters that had passed the Standard 53 cyst reduction test. The two filters were a precoat bag filter and a compressed carbon block depth filter. A carbon block filter was selected as the third unit. This filter was selected because it had produced carbon fines that had interfered with the current Standard 53 cyst reduction protocol. All units used flow-limiting devices to control the maximum flow rate.

B. Challenge Particles

The test dust used was 0 - 5 microns obtained from Particle Technologies. The polystyrene microspheres were obtained from Bangs Laboratories and were 2.967 micron mean diameter with a 0.037 micron standard deviation. They were dyed fluorescent orange to distinguish them from the fluorescent green of the stained oocysts. The *Cryptosporidium parvum* oocysts were obtained live from Waterborne, Inc. derived from calves.

C. Test Rigs

The test rig used for the challenge test was designed to run the test dust, microsphere and oocyst challenges on the same filter. The rig accommodated two filters of the same type to perform the testing in duplicate. The challenges were directly injected into the influent. The test rig operation procedure is located in Appendix B.

D. Influent Parameters

The influent water characteristics were maintained at the required levels throughout all tests. The test units were cycled at a 50% on/50% off, 10 minutes on filter A and 10 minutes on filter B.

E. Test Dust Loading

The test dust was set to provide $5 \pm 3 \times 10^4$ of 3 - 4 micron particles as the influent challenge providing a turbidity of 10 NTU. The test dust was used to load the filter media and produce a flow reduction as the filter clogged.

F. Sampling

The test procedure followed the Standard 53 protocol which had samples at the beginning of the fourth cycle, and at 25, 50, and 75 percent flow reduction. The microspheres and oocysts were collected at an additional sample point at the beginning of the eighth cycle. At the start of the test the microspheres and oocysts were introduced without test dust for a full eight cycles. At the end of the eighth cycle the microsphere and oocyst challenge was stopped and the test dust was introduced until the next sampling point. When this point was reached, the system was run for three cycles without challenge to reduce the test dust in the effluent. This reduced interferences in the counting of the challenge particles. The microsphere and oocyst challenge was then introduced for three cycles. The sample was taken at the beginning of the fourth sample when pressure was first introduced to the unit. This was used to simulate a sudden increase of pressure on the filter which could cause particle breakthrough.

G. Challenge Particle Analysis

The ANSI/NSF Standard 53 test dust was analyzed by particle counter as specified by the Standard. An epi-fluorescent microscope was used to count the influent and effluent concentrations of microspheres and oocysts.

The microsphere counting method (Appendix C) involved passing a measured volume of sample through a 47 mm 0.45 micron membrane filter and counting the microspheres under the microscope. Influent concentrations were determined by counting 10 random areas and factoring to determine the total count. Effluent concentrations were determined by counting the entire

filter.

The oocyst analysis (Appendix D) involved the filtering of a known volume of sample through a 25mm membrane filter and performing a direct antibody staining technique to stain the oocysts fluorescent apple-green. Ten milliliters of the influent samples were passed through the filter and the entire filter was counted. A one liter effluent sample was centrifuged, concentrated and passed through the filter. In all cases, the entire filter was scanned and counted.

A comparison between 25 mm and 13 mm membrane filters was performed to determine if a smaller filter would result in a more precise count. The sample preparation and counting techniques were compared by preparing seven filters from one influent sample. These filters were analyzed by three different analysts at two different laboratories.

III. Test Results

The test results are summarized in Tables 1 through 7.

A. Precoat Membrane Filter

The results for the precoat membrane filter are summarized in Table 1. The filters achieved a greater than 99.95% reduction of the microspheres. No *Cryptosporidium* oocysts were detected in the effluents, however a 99.95% reduction was not achieved at one sampling point due to a low influent challenge. This model of filter had previously passed the Standard 53 test dust reduction. The two filters that were used in this test passed the test dust reduction requirement of 99.95% at start-up but both of the filters failed to meet the reduction requirement at the 25%, 50%, and 75% flow reduction points. This model of filter had previously passed the Standard 53 test dust reduction.

B. Carbon Block Filter

The results for the carbon block filter are summarized in Table 2. This filter achieved a greater than 99.95% reduction at all of the sampling points for the microspheres, oocysts and test dust. No oocysts were observed in the effluents, however microspheres were observed in the effluents. Two microspheres were detected in the filter A effluent at the fourth cycle and also in the 25% sample for both filters. Four microspheres were observed in the effluents from both filters at the 50% and 75% sample points. The concentration of microspheres in the influent was higher than the oocyst concentration at all of the sampling points.

C. Carbon Block Filter with Interfering Fines

The test dust values were not obtained for this filter due to failure of the test equipment.

Both filter A and filter B passed the 99.95% reduction criteria at the fourth and eighth cycle test

points for the microspheres and oocysts. The microspheres and oocysts began passing through filter B at the eighth cycle. There were no particles detected in the filter A effluent at the eighth cycle test point. Both filters failed the reduction criteria at the 25% flow reduction sample point. Filter B failed at the remainder of the test points for microspheres and oocysts. Filter A failed the microsphere reduction at the 50% and 75% test points and the oocyst reduction at the 50% test point.

The lowest percent reduction for filter A was achieved at the 25% sample point. The percent reduction for both oocysts and microspheres increased for the 50% and again at the 75% test point for filter A. The percent reduction for filter B decreased throughout the test. Filter B also gave a lower percent reduction than filter A.

For both filters in this test the number of microspheres passed was greater than the oocysts for all test points, however the influent challenge was higher for the microspheres than the oocysts.

D. Comparison of Counts Between Analysts Using 25 mm and 13 mm Filters

The results for this comparison are summarized in tables 4, 5 and 6. This test was done to see if a smaller filter would give a more precise count. Replicate 7 for the microsphere analysis on the 25 mm filter gave an abnormally low results. This replicate was removed from the data analysis in table 7. For all of the analysts the confidence interval was smallest using the 25 mm filters.

This comparison was also done to test the reproducibility of sample preparation and analyst counting techniques. Seven filters were prepared from the same sample of influent water with agitation before each sample aliquot was taken. Each filter was then counted by three different analysts. Two of the analysts were from the same laboratory.

The 95% confidence interval for the microsphere and the *Cryptosporidium* counts are summarized in tables 4, 5 and 6. The 25mm filters gave a smaller 95% confidence interval for the microspheres and the oocysts. The 95% confidence interval for the microsphere analysis excluding replicate 7 ranged from 8.5% to 9.6% for the analysts. The confidence interval for the *Cryptosporidium* counts ranged from 12.6% to 19.2%.

Table 7 summarizes a comparison of counts between two analysts on effluent samples. This was done to analyze the results obtained from a lower count. Seven filters were prepared from the same sample of effluent with agitation before each sample aliquot was taken. Each filter was then counted by two different analysts. The average 95% confidence interval obtained for the microsphere effluent was 12.0%. The average confidence interval for the oocysts was 30.5%.

Table 1. Precoat Membrane Filter Test Data Summary

Sample Point	Influent particles	Filter A Effluent particles	Filter B Effluent particles	Filter A Percent Reduction	Filter B Percent Reduction
Precoat Filter Microsphere Test (counts in #/L)					
4th cycle	39700	<2[1]	<2	>99.995	>99.995
8th cycle	28500	<2	<2	>99.993	>99.993
25% flow red.	21300	<2	<2	>99.991	>99.991
50% flow red	63700	2	<2	99.997	>99.997
75% flow red.	90000	12	2	99.987	99.998
Precoat Filter Live *Cryptosporidium parvum* Oocyst Test (counts in #/L)					
4th cycle	42000	<2	<2	>99.995	>99.995
8th cycle	1360	<2	<2	>99.85	>99.85
25% flow red.	17000	<2	<2	>99.988	>99.988
50% flow red	53600	<2	<2	>99.996	>99.996
75% flow red.	37700	<2	<2	>99.995	>99.995
Precoat Filter ANSI/NSF Standard 53 Test Dust (counts in #/ml)					
Start up	58500	1.5[2]	5.4	99.997	99.991
25% flow red.	53100	398	296	99.25	99.443
50% flow red	60800	278	269	99.543	99.558
75% flow red.	68400	71	68	99.896	99.901

[1]The effluent samples were split to analyze for microspheres and oocysts in the same sample. This increased the detection limit from <1 to <2.

[2] The test dust counts were obtained using a particle counter which provides an estimate of the concentration that may not be a whole number.

Table 2. Carbon Block Test Data Summary

Sample Point	Influent particles	Filter A Effluent particles	Filter B Effluent particles	Filter A Percent Reduction	Filter B Percent Reduction
Carbon Block Microsphere Test (counts in #/L)					
4th cycle	69500	2	<2	99.997	>99.997
8th cycle	79800	<2	<2	>99.997	>99.997
25% flow red.	55200	2	2	99.996	99.996
50% flow red	70700	4	4	99.994	99.994
75% flow red.	59700	4	4	99.993	99.993
Carbon Block *Cryptosporidium parvum* Oocyst Test (counts in #/L)					
4th cycle	40000	<2	<2	>99.995	>99.995
8th cycle	52800	<2	<2	>99.996	>99.996
25% flow red.	50900	<2	<2	>99.996	>99.996
50% flow red	35800	<2	<2	>99.994	>99.994
75% flow red.	43100	<2	<2	>99.995	>99.995
Carbon Block ANSI/NSF Standard 53 Test Dust (counts in #/ml)					
Start up	60800	3.4	1.2	99.994	99.998
25% flow red.	60000	6.0	<1	99.990	>99.998
50% flow red	74200	4.3	<1	99.994	>99.999
75% flow red.	55800	<1	<1	>99.998	>99.998

Table 3. Carbon Block Filter With Interfering Fines Test Data Summary

Sample Point	Influent particles	Filter A Effluent particles	Filter B Effluent particles	Filter A Percent Reduction	Filter B Percent Reduction
Carbon Block Filter Microsphere Test (counts in #/L)					
4th cycle	27000	<2	<2	>99.993	>99.993
8th cycle	24800	<2	2.1	>99.992	99.992
25% flow red.	43200	473	859	98.905	98.012
50% flow red	65000	127	2841	99.805	95.629
75% flow red.	62200	353	3558	99.433	94.280
Carbon Block Filter Live *Cryptosporidium parvum* Oocyst Test (counts in #/L)					
4th cycle	22000	<2	<2	>99.991	>99.991
8th cycle	19000	<2	4	>99.990	99.979
25% flow red.	19600	21	42	99.893	99.786
50% flow red	29600	21	740	99.929	97.500
75% flow red.	24400	11	1155	99.955	95.266

Table 4 Comparison of Counts Between Three Analysts Using 13mm Filters

	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Replicate 7	Average	Confidence Interval at 95%
Microspheres									
Analyst 1	26700	26400	24700	31400	28200	23000	32700	27586	27586±2577 (9.3%)
Analyst 2	27400	26400	25000	35500	31200	23100	33000	28800	28800±3353 (11.6%)
Analyst 3	21200	20900	17500	30900	23900	22100	26900	23343	23343±3262 (14.0%)
Oocysts									**Average 26576±3064 (11.5%)**
Analyst 1	16400	21400	12000	12800	22000	19200	11000	16400	16400±3390 (20.7%)
Analyst 2	10800	20000	9800	12200	18600	16200	12000	14229	14229±2975 (20.9%)
Analyst 3	9200	14800	11000	11400	18800	12600	11400	12743	12743±2345 (18.4%)
									Average 14457±2903 (20.1%)

Table 5 Comparison of Counts Between Three Analysts Using 25mm Filters

	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Replicate 7	Average	Confidence Interval at 95%
Microspheres									
Analyst 1	63700	58000	54000	57000	57000	73000	14200	53843	53842±13770(25.6%)
Analyst 2	66000	68000	58000	58000	61000	75600	13500	57157	57157±14994(26.2%)
Analyst 3	62000	53500	50600	50300	48400	63900	11500	48600	48600±12906(26.6%)
Oocysts									**Average 53200±13890(26.1%)**
Analyst 1	53600	34400	59100	42600	44900	45200	50800	47229	47229±5971 (12.6%)
Analyst 2	56400	28600	60700	38500	52600	48400	56000	48743	48743±8446 (17.3%)
Analyst 3	46000	17400	41600	37600	33700	33400	33900	34800	34800±6669 (19.2%)
									Average 43591±7029 (16.1%)

Table 6 Comparison of Microsphere Counts on 25 mm Filters Removing Slide 7

Analyst 1	63700	58000	54000	57000	57000	73000		60450	60450±5538(9.2%)
Analyst 2	66000	68000	58000	58000	61000	75600		64433	64433±14994(8.5%)
Analyst 3	62000	53500	50600	50300	48400	63900		54783	54786±12906(9.6%)
									Average 59889±5422 (9.1%)

Table 7. Comparison of Counts Between Two Analysts on Effluent Samples

	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Replicate 7	Average	Confidence Interval at 95%
Microspheres									
Analyst 1	372	370	338	338	422	484	304	375	375±45 (11.9%)
Analyst 2	362	406	368	326	462	486	323	390	390±47 (12.1%)
Oocysts									**Average 383±46 (12.0%)**
Analyst 1	88	146	82	114	56	60	154	100	100±29 (29%)
Analyst 2	78	136	94	108	50	56	158	97	97±30 (30.5%)
									Average 98.5±30 (30.5%)

IV. Discussion

A. Precoat Filter

This precoat filter model had previously been tested for Standard 53 test dust reduction and had passed the 99.95% reduction criteria. The set of filters that were used in this study did not pass the reduction criteria for test dust. The 25% flow reduction test point gave the lowest percent reduction of the test dust influent at 99.25% for filter A and 99.44% for filter B. The percent reduction increased as the filter was loaded with test dust.

The filters met the reduction criteria for the removal of microspheres at all of the sampling points. As the pressure drop across the filter media increased more microspheres passed through the filter. This is the opposite response from the test dust. The data seems to indicate that the test dust reduction does not occur by the same physical mechanism as the microsphere reduction on this filter. The test dust particles could be binding to each other or the filter by an electrostatic charge or some other effect that would increase the filtration efficiency of the filter as the test dust loading increases. The microspheres do not react with the same process and are passed by the filter as the test dust loading and the pressure drop increases.

No *Cryptosporidium* oocysts were observed in the effluent. The filters passed the percent reduction criteria at all of the test points except at the eighth cycle. At the eighth cycle the influent concentration was too low to achieve the 99.95% reduction. The influent concentration was 1360 oocysts/L. In order to reach the 99.95% reduction, 0.68 oocysts/L would have to be observed in the effluent. The limit of detection for the microscopic counting method was 2 oocysts/L. This example illustrates why a lower limit for the influent challenge will have to be set. This limit will have to take into account the limit of detection for the counting method.

On this filter the microspheres were more conservative than the oocysts. No oocysts were detected in the effluent so the removal of the oocysts in relation to the pressure drop could not be determined for this filter.

This set of filters also illustrated that the current Standard 53 test dust reduction method provides a conservative estimation of the filtration efficiency for *Cryptosporidium* removal when compared to live oocysts. The test dust on these filters did not pass the reduction criteria but no oocysts were observed in the effluents.

B. Carbon Block Filter

This filter passed the 99.95% reduction criteria for microspheres, *Cryptosporidium* and test dust at all of the sampling points.

At the 25% sampling point 2 microspheres/L were observed in the effluent for each filter. At the 50% and 75% test points 4 microspheres/L were observed. The microspheres broke through this filter while the oocysts did not as was observed with the precoat filter.

No oocysts were observed in the effluent as in the precoat filter test. Since no oocysts were observed a relationship between pressure drop and oocyst removal cannot be established.

A few test dust particles were observed in the effluent at a low level. This level was too low to establish any significance to the observed values.

C. Carbon Block Filter with Interfering Fines

Test dust data was not acquired for this filter test due to equipment failure.

This filter had not passed the Standard 53 test dust reduction. It was thought that this failure resulted from fines released from the carbon block and recorded as test dust particles. These fines did not interfere with the counting of the microspheres and oocysts since they were stained with fluorescent dyes.

The filters did not pass the reduction criteria at the 25%, 50% and 75% flow reduction test points for the removal of microsphere. The two filters did not produce similar results. Filter A achieved its lowest percent reduction for microspheres at the 25% test point while Filter B produced its lowest reduction at the 75% test point. The filter A effluent contained significantly fewer microspheres than the filter B effluent at all of the test points. A similar effect was observed for the *Cryptosporidium* removal. Filter A met the *Cryptosporidium* removal criteria at the 75% flow reduction test point while Filter B had a 95.266% removal. This difference in results between the two filters illustrates why two filters should be tested.

The percent reduction for Filter B decreased as the pressure drop increased. This effect was observed for both the oocysts and microspheres indicating that their removal may involve the same mechanism. This conclusion could not be drawn from Filter A which did not follow a consistent pattern for the microsphere and oocyst reduction.

The microspheres achieved a lower percent reduction than the oocysts except at the eighth cycle test point. At the eighth cycle the number of microspheres and oocysts observed was very small and both filters passed the reduction criteria at this point. The smaller percent reduction produced by the microspheres show that they would be a conservative surrogate.

D. Microbiological Methods

The automatic counting method was not done due to problems with the calibration of the instrument.

Seven replicate membrane filters were prepared from one sample to validate the complete

microbiological counting methods. A comparison was made on the precision of counts obtained using a 25 mm membrane filter and a 13 mm membrane filter. One of the filters from the 25 mm microsphere analysis was found to be low. It was excluded from the data analysis. It was thought that a 13 mm filter would give greater precision since there is less area to count. This was found to be incorrect. The 25 mm filter produced a count with greater precision for both microspheres and oocysts. The actual count produced on the 25 mm filters was also greater than that on the 13 mm filters. Upon further investigation it appeared that the 13 mm filters did not fit the filtration apparatus correctly and some of the sample may have been bypassing the filter.

Each filter was counted by three analysts. A significant difference was found between one or more of the filters and between one or more of the analysts for both the microsphere and oocyst counts. The confidence interval for each of the analysts was then obtained to find the precision of the method. The microsphere method, using 25 mm filters, produced an average confidence interval of 9.1% for the three analysts. The *Cryptosporidium* method, using 25 mm filters, produced an average confidence interval of 16.1% for the three analysts. This gives an estimate of the precision of the method. To obtain a true interlaboratory value for the method the filters should have been prepared by each analyst. This was not done due to the difficulty in shipping the sample. Error would have been introduced by not preparing the filters immediately after sampling since the oocysts may adhere to the sample container. The microsphere analysis for the 25 mm filters should have been redone since an outlier was found.

The precision of the influent concentration determination creates an interesting situation regarding the percent reduction. The precision for calculating the *Cryptosporidium* influent concentration was 16.1%. This situation is summarized in the following table.

Table 10. Percent Reduction Values

	True Value - 16.1%	**True Value**	**True Value + 16.1%**
Influent Value	41,950	50,000	58,050
effluent value to give 99.95% reduction	21	25	29
effluent value to give 99.96% reduction	17	20	23

One possible solution to ensure that the reduction criterea is met is to set the reduction criterea to 99.96% which would provide a conservative reduction value as illustrated in Table 10. Another solution would be to require that no oocysts would be detected in the effluent since it appears that a non-detect value is what will be used in regulation.

E. Particle Counting

The on-line particle counter showed a spike in the number of particles released by the filters when the flow was switched from filter to filter. The grab samples that were taken for the microscopic analysis included this spike. The data did not indicate that there was any unloading of the filters at any other time during the testing.

The on-line particle counter did not remain synchronized with the switching of the influent from filter A to filter B. This made it very difficult to produce a comparison between the microscopic counting methods and the particle counter.

V. Strengths, Deficiencies and Recommendations

This test study acquired additional data for the validation of a test method for the removal of *Cryptosporidium parvum* oocysts from water by drinking water treatment unit filtration. This study provided the following additional information:

A. Strengths of the Study

a) The test rig enabled a comparison of *Cryptosporidium* and microsphere removal to be done on the same filter eliminating error caused by manufacturing differences.

b) The testing showed the importance of maintaining the influent challenge to evaluate the percent reduction.

c) The particle counting data indicated that a higher concentration of particles were released from the filters at flow switching points. The particle counter also showed that no other releases occurring in the test cycle.

d) The data for the carbon filters with fines gave differing results for each filter. This illustrated the importance of testing more than one filter.

e) The 2.967 micron microspheres were a more conservative challenge than the *Cryptosporidium* oocysts for the filters in this test.

B. Deficiencies

a) Replicate testing was not performed at the pass/fail limit of 25 particles/Liter.

b) A statistically significant number of filters of the same model were not tested due to cost and time restraints.

C. NSF Recommendations

a) Proceed with the introduction of live *Cryptosporidium* oocyst testing protocol into Standard 53 with the following provisions:

1) Test two filters of the same model to account for manufacturing differences.

2) Set the acceptance criteria to 'no oocysts detected' in the effluent to provide a safety factor due to the limits of the current microbiological methods.

b) Use the 2.967 micron microsphere as a surrogate using a 99.95% reduction criteria. This reduction criteria would be used since the microspheres are a more conservative challenge than the oocysts. Also, the confidence interval for the counting of the microspheres is smaller than that of the oocysts.

VI. Items for Task Group Discussion

The Task Group should include the following points in a discussion of the testing protocol:

a) The size of the microspheres. Rationale for establishing the size of the microspheres.

b) The acceptance criteria. The precision of the enumeration methods must be discussed in setting the requirements for the acceptance criteria.

