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American National Standard*

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Public Drinking Water Equipment Performance - Filtration



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NSF International Standard/
American National Standard
for Public Drinking Water
Equipment Performance –

**Public Drinking Water
Equipment Performance
– Filtration**

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Foreword²

The purpose of this Standard is to establish minimum performance requirements for filtration devices used in the treatment and production of public drinking water. The Standard describes the performance evaluation test procedure for the product specific challenge testing of full scale ultrafiltration (UF) and microfiltration (MF) membrane modules, bag filters, and cartridge filters for the removal of microbial contaminants. It 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 W³. The procedures in this Standard have been adapted from and are consistent with those applications described in the EPA's Membrane Filtration Guidance Manual (MFGM)⁴. Quality assurance/quality control procedures are also described under informational annexes to ensure that data generated from the testing will provide sound analytical results that can serve as the basis for the performance evaluation.

It is anticipated that alternative filtration technologies may be addressed under this Standard in the future.

This Standard was developed by the NSF Joint Committee on Public Drinking Water Equipment Performance with balanced input from industry, regulatory, and end-user groups using the consensus process described by the American National Standards Institute. The Standard incorporates NSF International's nearly two decades of experience managing the U.S. Environmental Protection Agency's Environmental Technology Verification Drinking Water Systems Center (ETV DWSC).

Suggestions for improvement of this Standard are welcome. This Standard is maintained on a Continuous Maintenance schedule and can be opened for comment at any time. Comments should be sent to Chair, Joint Committee on Public Drinking Water Equipment Performance at standards@nsf.org, or c/o NSF International, Standards Department, P.O. Box 130140, Ann Arbor, Michigan 48113-0140, USA.

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³ Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402 <www.gpo.gov>.

⁴ U.S. Environmental Protection Agency (USEPA), Office of Water, Washington, D.C. 20460 <www.epa.gov>

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NSF/ANSI 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 for 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 this 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*⁵

APHA, *Standard Methods for the Examination of Water and Wastewater*, twentieth edition⁶

ASTM D6908-03 *Standard Practice for Integrity Testing of Water Filtration Membrane Systems*⁷

ASTM E29-02 *Standard Practice for Using Significant Digits in Test Data to Determine Conformance with Specifications*⁷

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 typically greater than 1 µm 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.

⁵ Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402 <www.gpo.gov>.

⁶ American Public Health Association (APHA), 800 I Street, NW, Washington, DC 20001 <www.apha.org>.

⁷ ASTM International, 100 Barr Harbor Drive, West Conshohocken, 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 as 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., $\log_{10}(\text{feed concentration}) - \log_{10}(\text{filtrate concentration})$).

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 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% 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 $3.00 \pm 0.15 \mu\text{m}$. 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

The test dust is used to load the filter to create a pressure drop across the filter. Test dust 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.

NOTE – Sanitization should be done in accordance with the manufacturer's recommended procedures or a commonly accepted practice such as AWWA C653 - *Disinfection of Water Treatment Plants*.

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 $50\% \pm 5\%$ 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 minute 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.

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	

5.9.3 Flow rate

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

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

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 of 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 – 5×10^5 to 3.16×10^6 plaque forming units per milliliter (PFU/mL); and
- *B. atrophaeus* – 5×10^5 to 3.16×10^6 colon forming units (CFU) per 100 mL.

NOTE – The MFGM calls for the maximum challenge concentration to be $6.5 \log_{10}$ above the organism's detection limit (3.16×10^6). 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 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.

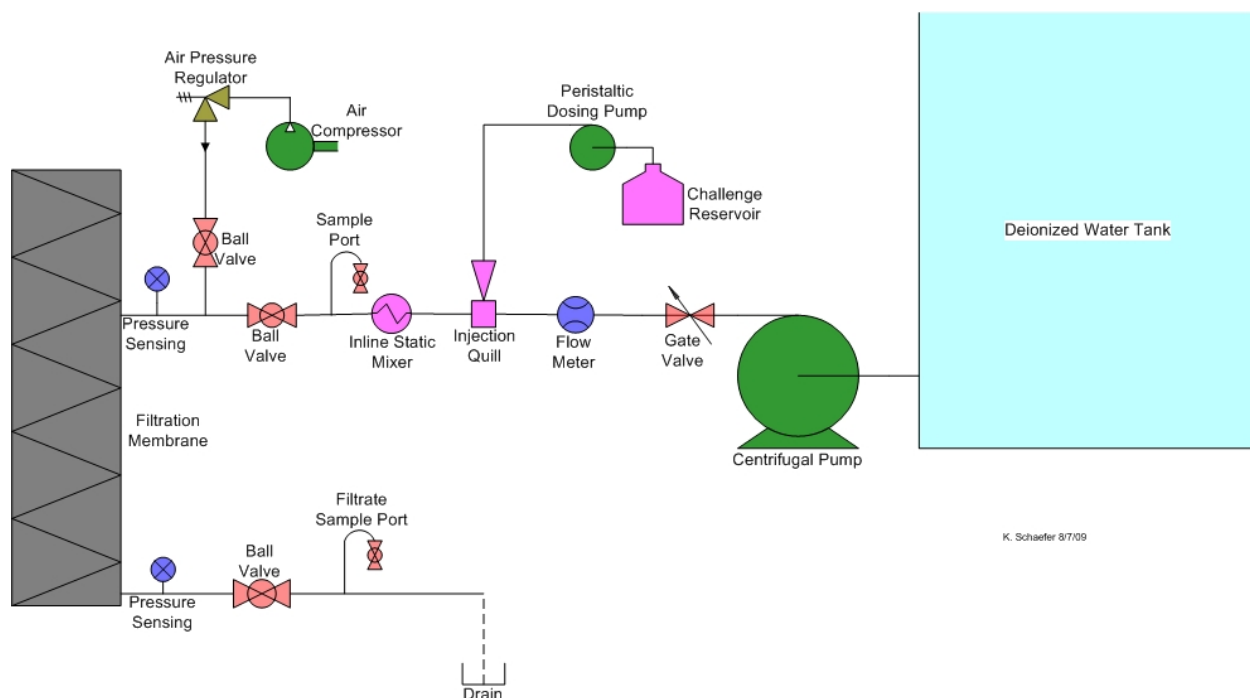


Figure 3 – Example test apparatus

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% 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 < 0.3 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 with an appropriate disinfectant.

NOTE – Sanitization should be done in accordance with the manufacturer's recommended procedures or a commonly accepted practice such as AWWA C653 - *Disinfection of Water Treatment Plants*.

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
<i>B. atrophaeus</i> 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 mL volumes of the feed samples shall be serially diluted for enumeration. One mL 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 24 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 log₁₀ 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 log₁₀ 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 log₁₀ concentration.

– *Method 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.