World Health Organization

WHO International Scheme to Evaluate Household Water Treatment Technologies

General Testing Protocol #11: Gravity Flow Mechanical Filtration Batch System Technology Seal Integrity

Geneva, Switzerland

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1. PRODUCT INFORMATION REQUIREMENTS

The manufacturer is to provide detailed product information as required in the Expression of Interest (EoI) which is located on the WHO website at: http://www.who.int/household_water/scheme/en/. This information is to include the basic information necessary to identify the product and conduct the testing according to the manufacturer's use instruction, which may include, but not limited to:

Flowing system (gravity fed batch stand alone):

Flow rate

Volumetric capacity

Power requirements

Operating pressure

Maximum operating pressure

Operation instructions – to include: assembly, conditioning, and use instructions, daily operation and maintenance, replacement components, cleaning, backwashing and short term storage instructions (if any).

Manufacturer capacity and supporting information upon which capacity is based.

Designated Test Laboratory

The designated testing laboratory shall be identified.

2. PURPOSE

The household water treatment (HWT) product shall be evaluated for seal integrity as microbiological performance based on recommendations and testing principles set forth in the World Health Organization's *Evaluating Household Water Treatment Options: Health-based targets and microbiological performance specifications* (2011). Testing conducted by one of the WHO designated testing laboratories shall also be done in line with the terms and conditions outlined in the WHO International Scheme to Evaluate Household Water Treatment Technologies Procedure ("Procedure"). The Procedure can be found on the aforementioned WHO website.

3. METHOD

3.1. Replicate samples

Three (3) production units shall be selected and run as triplicates (3) in one (1) test water.

3.2. Test waters

Test water shall be prepared daily. An important aspect is that testing will be simulated to model actual field and use conditions. One (1) type of water will be used: a general test water (GTW) representing high quality groundwater or rainwater. Table 1 provides the required test water characteristics and adjustment materials. Following test water preparation, total residual chlorine, pH, turbidity, temperature, total dissolved solids (TDS), and alkalinity shall be measured and reported on the test water tank. TOC is verified during test water prep as the weight of the adjustment material addition. Sufficient volume shall be collected to allow for a retain volume for back-up analysis, if needed. The following methods, or equivalent, shall be used:

- Chlorine (total): SM 4500-Cl G or UNE-EN ISO 7393-1
- pH: SM 4500 H+ B
- Turbidity: EPA 180.1
- Temperature: SM 2550
- TDS: SM 2540C
- Alkalinity: SM 2320-B
- TOC: humic or tannic acid addition to the test water volume is to be weighted out based on the carbon content of the humic or tannic acid and is calculated to be within the test water specification range. As an alternate, SM 5310C, in water (GTW, lower TOC); SM 5310B, in water (CTW, higher TOC) may be used.

3.2.1. General test water

The general test water represents non-stressed phase of testing. Reverse osmosis treated water shall be used as the base water and adjusted to meet the following characteristics:

Table 1: General Test Water characteristics

Constituent	Specification	Adjustment Materials (CAS#[3])
Chlorine[1] (mg/L)	< 0.05	None
pH	7.0 ± 0.5	Inorganic acid or base: Hydrochloric acid (7647-01-0) Sodium hydroxide (1310-73-2)
TOC (mg/L)	1.05 ± 0.95 mg/L	Tannic acid (1401-55-4, Supplier: Alfa Aesar)
Turbidity (NTU)	< 1 NTU	No adjustment
Temp (°C)	20 ± 3°C	Not applicable
TDS (mg/L)	275 ± 225 mg/L	Sea Salts, Sigma Chemical Company (7732-18-5)
Alkalinity[2] (mg/L as CaCO3)	100 ± 20 mg/L	Sodium bicarbonate (144-55-8)

[1] All chlorine shall be removed to below detection limits without the aid of added chemical(s) and is commonly accomplished by using activated carbon. Chlorine shall be measured prior to addition of test water adjustment materials. Chloride levels in Challenge Water may cause interference with analytical technique; measurements shall be made prior to addition of sodium chloride.
[2] Intended to buffer pH. Analyzed values may deviate from this range.
[3] Chemical Abstract Service registration number. Refer to the definition section of this document for additional information.

3.3. Microbiological Organisms and Challenge Concentrations

Table 2 shows the organisms and American Type Culture Collection numbers (ATCC) used in evaluating seal integrity as microbiological performance. The target pretreatment concentrations shall be sufficient to demonstrate: *highly protective, protective, or limited protection.*

Table 2: Microbiological organisms and reduction requirements

Organism	Pretreatment Challenge[1]	Minimum Required Reduction (log)	
		Highly Protective	*Protective or Limited Protection*
Virus[2,3]: MS-2 coliphage (ATCC 15597-B1, with host organisms: *E. coli* (ATCC 15597) or *Salmonella typhimurium* (WG49 NCTC 12484) and phiX-174 coliophage (ATCC 13706-B1) with host organisms: *E. coli* (ATCC 13706 or ATCC 700078)	$\geq 10^8$/L	≥ 5	≥ 3

[1] The pretreatment challenges may constitute greater concentrations than would be anticipated in source waters, but these are necessary to properly test, analyze, and quantitatively determine the indicated log reductions. The pretreatment challenge must not be less than that required to demonstrate the geometric mean and standard deviation minimum required reduction described below.
[2] Virus performance claim will be based on the poorest log reduction of the two phages.
[3] Host selection is dependent on method. Refer to Section 3.3.1.4 Organism Methods.

3.3.1. Selection of Microorganisms

For seal integrity for mechanical technologies seeking *highly protective* or *protective*, testing shall only include the surrogates for enteric virus performance.

3.3.1.1. Enteric Virus

Human enteric viruses are the smallest pathogens, making them more difficult to remove by physical processes, such as filtration. Specific viruses may be less sensitive to disinfection than enteric bacteria and some protozoan parasites. Using human or animal viruses in laboratory testing is complicated, expensive and given the availability of comparable surrogates, this later option was chosen for the Scheme. Two different surrogate bacteriophages, MS-2 and phiX-174, shall be used to evaluate the performance of HWT products for performance. In choosing surrogates, consideration included the wide variety of different viruses' resistance to potential treatment processes that enteric viruses vary greatly in terms of size, isoelectric points, type of nucleic acid, presence of lipids, and the structure of the proteins in the capsid. Additionally, some treatment systems have more than one mechanism that would remove/inactivate viruses. For example, a filtration system (activated carbon) may be combined with a UV light system. Some viruses may be more easily removed by adsorption to the activated carbon than others, and others may be more resistant to the UV light. For these reasons and due to not using an actual pathogen, the testing of two bacteriophages, with varying characteristics and responses to treatment processes, shall be used in the assessment of the performance of HWT products.

MS-2 and phiX-174 are extensively used bacteriophages as models for human enteric virus removal by water treatment processes. A great deal is known about the resistance of these bacteriophages to disinfectants. They are easy to grow to large number. Both are similar size and lack a lipid coat like many of the human enteric viruses.

MS-2, 24nm in diameter, is a singled stranded RNA virus, with a low isoelectric point (3.9). A

low isoelectric point makes it less sticky (adsorbs to a lesser degree) than poliovirus and has been used as a conservative model for removal by adsorption processes. MS-2 is very resistant to inactivation by low-pressure UV light and has been used as a model virus to measure UV light dose in UV light reactors (collimated beam). It is one of the more hydrophobic non-lipid containing viruses.

PhiX-174, 25-27nm in diameter, is a single stranded DNA with an isoelectric point of 6.6. It is less hydrophobic than MS-2. Research suggests that it is more resistant to halogen disinfectants like iodine and chlorine dioxide than MS-2.

3.3.1.2. Organism methods

Production and assay procedures for the microbial challenges and equivalent methods shall include, but not be limited to:

- **Coliphage MS-2** (ATCC 15597-B1) shall be prepared and assayed using:
  - The method in Annex A, Section A.8.2.2 of NSF/ANSI 55: Ultraviolet Microbiological Water Treatment Systems (2012); *E. coli* host ATCC 15597; or
  - NEN-EN-ISO 10705-1 (Detection and enumeration of bacteriophages Part 1: Enumeration of F-specific RNA bacteriophage).
    - *Salmonella typhimurium (WG49)* host NCTC 12484 or *E.coli* host ATCC 15597. Analyses shall be conducted in triplicate; the geometric mean and standard deviation for each water type and across all water types examined shall be reported.
    - Samples shall be stored at a temperature between 1 – 8°C and processed within 24 hours of collection.
    - Required sample volume to allow for processing in triplicate and a retain volume: 12ml
- **Coliphage phiX-174** (ATCC 13706-B1) shall be prepared and assayed using:
  - The method in Annex A, Section A.8.2.2 of NSF/ANSI 55: Ultraviolet Microbiological Water Treatment Systems (2012); *E. coli* (host) ATCC 700078; or
  - NEN-EN-ISO 10705-1 (Detection and enumeration of bacteriophages Part2: Enumeration of somatic coliphages)
    - *E. coli* host ATCC 700078 or ATCC 13706
    - Analyses shall be conducted in triplicate; the geometric mean and standard deviation for each water type and across all water types examined shall be reported.
    - Samples shall be stored at a temperature between 1 – 8°C and processed within 24 hours of collection.
    - Required sample volume to allow for processing in triplicate and a retain volume: 12 ml.

3.4. Other Test Details

3.4.1. Untreated control

The microbiologically spiked test water to be used as the influent challenge concentration, shall also serve as the untreated control. See Table 2 for concentrations.

3.4.2. Blank sample

Prior to test initiation, using the GTW, the product shall be tested for the presence of the test

organisms without microbiological addition to confirm that the product arrived to the laboratory free of test organisms. Systems shall flow sufficient volume of GTW, with no microbiological addition, through the system challenge to allow for the collection of the necessary volume for analysis for the organisms of Table 2.

3.4.3. Quality assurance/quality control (QA/QC)

The testing laboratory will adhere to the requirements of their QA/QC procedures and ISO 17025 requirements and must be able to provide documentation of adherence, which are to include but not be limited to quality checks on organism stocks, calibration of instruments, testing environmental controls, etc.

3.4.4. Product residual or wetted contact material of concern

For products that employ a disinfectant component, the component will be removed from the treatment for the seal integrity testing.

If the data does not already exist, an additional test, separate from the seal integrity testing, to investigate the disinfectant residual may be required. The active agent residual shall not constitute a threat to health. The WHO Guidelines for Drinking-water Quality (2011) shall be used to determine acceptable levels in the product water.

3.4.5. Microbiological sample points

Sampling for microbial pathogens shall be conducted according to the schedule shown in Table 4. Seeding shall be required for microbiological challenge points.

- Seeding shall be used to purge the system of the uncontaminated water with a sufficient flow of contaminated test water (seeding). The systems shall be exposed to a minimum of 10 units void volumes or 1L, whichever is greater, of microbiologically challenged water per Table 2 immediately prior to sample collection and continued through sample collection.
- A ‘sampling event’ includes seeding and sample collection.
- For batch systems, a full batch shall be used for seeding and the final 500 ml collected and sub-sampled into prepped bottles for microbiological analysis. Additional full batches may be used if seeding or sample collection volume requires additional volume.

Sample Collection for GTW:

- 1 blank sample analyzed for the organism of Table 2
- 1 pretreatment sample analyzed for organism of Table 2
- 1 sample for Test Water Characteristics
- 3 post-treatment samples (1 per unit) analyzed for organism of Table 2

3.4.6. Conditioning

There shall be no microbiological addition during conditioning, and the volume used for conditioning shall not be counted as accumulated volume in determining test volume.

3.4.7. Cycling

Cycling shall coincide with batch processing, unless product use instructions are such that a different approach. Testing shall be according to the use instructions.

3.4.8. Daily test capacity

Test capacity shall be sufficient volume for seeding and sampling. For batch systems, this would be a single full batch, unless this volume does not allow for sufficient seeding and sampling. At which time an additional batch(es) would be required.

3.4.9. Leakage test

Flowing systems shall not leak during test operation. Any leaking during test operation shall be recorded in the laboratory bench sheets.

3.4.10. End of test

Collection of effluent samples following a single seeding or a full batch.

3.4.11. Log reduction calculation

Testing shall be conducted simultaneously on the test dictated number of replicates. At each microbiological sampling point, pretreatment/influent and post-treatment/effluent water samples shall be collected and each analyzed in triplicate.

When reporting the geometric means of the triplicate counts, if all three counts are non-detect for the organism, the geometric mean should be reported to indicate "Less than" (<). In the event one or more PFU, CFU, or oocysts are found in one or two of the triplicate counts, the "less than" counts are to be treated as being at the detection limit for the purpose of calculating the geometric mean and standard deviation.

Log reductions for the purpose of compliance with this test plan shall be calculated at each sample point as follows:

The geometric mean (GM) of each triplicate analysis (X) shall be calculated for each pretreatment/influent sample and replicate post-treatment/effluent as:

$$[1] \qquad GM = (X_{1*}X_{2*}\ldots X_n)^{(1/n)}$$

The geometric mean is defined as the *n*th root (where n is the count of numbers) of the product of the numbers. Such as, the geometric mean of the three numbers is the cube root of their product.

The geometric mean applies only to positive numbers. It is also often used for a set of numbers whose values are meant to be multiplied together or are exponential in nature, such as data that will be reported for the microbiological concentration in the pretreatment/influent and post-treatment/effluent waters of the testing.

Example of calculating the geometric mean:

Use triplicate post-treatment/effluent analyses results of: 1.00E+02, 7.70E+01, and 9.30E+01.

Since there are 3 numbers, the n-th root is the 3rd root. The geometric mean would be:

$$(1.00E+02 * 7.70E+01 * 9.30E+01)\text{^}1/3 = 8.95E+01$$

The log reduction for each replicate at each sample point shall be calculated using the results from [1], shown below as the negative $\log_{10}$ of the GM of each replicate post-treatment/effluent, GM_{eff}, divided by the GM of the pre-treatment/influent, GM_{inf}.

$$[2] \qquad \text{Log Reduction} = -\log_{10}(GM_{eff}/GM_{inf})$$

Example of calculating the log reduction:

Using an example pretreatment/influent geometric mean of 2.07+E8 units (such as CFU/100mL) and using the above examples reported geometric mean of the triplicate analysis of 8.95E+01 units (CFU/100mL), the log reduction would be:

$$Log_{10}\ 2.07+E8 - Log_{10}\ 8.95E+01 = \text{log reduction}$$

$$8.31 – 1.95 = 6.36 \text{ log reduction}$$

For reporting purposes, two (2) significant figures shall be reported. For the above example, 6.4 would be reported. For evaluation of log reduction against the pass/fail criteria, ASTM Standard E29 Absolute method shall be used, which does not allow for rounding.

3.5. Acceptable reduction deviation

Three (3) production products for three (3) lots of the product must continuously meet or exceed the reduction requirements shown in Table 2.

Each phage is treated separately and the result for the virus log reduction for seal integrity will be based on the phage for which the product performed the poorest. Additionally, the geometric mean of all microbiological reductions must meet or exceed the requirements of Table 2. Compliance with the requirements shall be based on the reduction percentage calculation.

3.6. Records

All pertinent procedures and data shall be recorded and provided in a final report. The general test plan for the product type provides a list of the data that is to be reported.

3.7. Completeness

Completeness is a measure of the number of valid samples and measurements that are obtained during a test period. Completeness will be measured by tracking the number of valid data results against the specified requirements in the test plan.

Completeness will be calculated by the following equation:

$$\text{Percent Completeness} = (V / T) \times 100\%$$

Where:

V = number of measurements that are valid

T = total number of measurements planned in the test

The specification for this data quality objective will be to achieve minimum 90% completeness for microbiological and disinfectant residual samples scheduled in the test plan or one (1) incomplete measurement (if less than 10 are taken).

4. PROCEDURE

Test waters shall be prepared daily and verified in accordance with Table 1. Daily test water characteristics shall be sampled, analyzed and results provided in the final report. All sample volumes collection, both microbiological and chemical shall be collected such that sufficient sample volume remains after analysis to allow for retain sample. The remaining volume of sample shall be retained for confirmation or retesting purposes, when necessary. Flows shall be measured and recording at each test day start, at sample collection (start), and at the end of each test day.

Table 3. Sampling Schedule

Test Day: Collection point	Test Water	Microbiological Tests	
Day 1: Following seeding as the last ~500ml of a full batch[1]	GTW	Influent	Effluent

[1] Refer to Section 3.4.8 Daily test capacity for information on products which require more than a single batch volume.

Procedure

1. Three systems (3) shall be tested simultaneously.
2. Systems shall be conditioned according to the product use instructions.
3. General test waters shall meet the characteristics of Table 1.
   a. Daily test water characteristics shall be sampled, analyzed and results provided in the final report.
   b. Sufficient sample volume shall be collected to allow for a retain volume. One set shall be used for analysis and reporting. The second sample shall be retained for confirmation or retesting purposes, when necessary.
4. Devices shall be operated according to the product's use instructions.
   a. Test plan permitted system cleanings or backwashes and/or component replacements shall occur as dictated by operator manual/use instruction requirements.
   b. The laboratory technician(s) shall note daily starting and ending flow rates, flow rates at sample collection, and any cleaning procedures, and other significant event throughout the testing.
5. Testing shall be according to the schedule of Table 4.
   a. Devices shall be operated based on the product use instructions.

b. At each microbiological challenge point, the following shall occur:

   i. Microbiologically spiked challenge water shall be prepared to meet the concentrations of Table 2. MS-2 and PhiX-174 are compatible for combined challenge.

   ii. Seeding and sample collection shall occur as:

      1. A complete batch of microbiologically spiked (Table 2) test water shall pass through the system, with the final 500ml collected for sample and analysis.

      2. If a single batch is less than 1L or does not be exposed the system to a minimum of 10 units void volumes of microbiologically challenged water, additional batches shall be passes to achieve seeding and sample collection requirements.

   iii. A sample shall be collected from the influent challenge and analyzed to confirm influent concentrations.

      1. As the influent challenge water for all units was from a single source of microbiologically spiked prepped test water, a single influent sample shall be taken and for analyzed for the organisms of Table 2.

      2. The influent microbiological shall be collected immediately from the microbiologically spiked test water after all effluent samples have been collected. A duplicate volume shall be collected and retained as a backup for confirmation or retesting purposes, when necessary.

   iv. Microbiological influent and effluent concentrations shall be presented in the final report.

5. DEFINITIONS

The following establishes definitions for terminology used with household water treatment as point-of-use or point-of-collection disinfectants or units and related components. This list is general for all Generic Test Plans (GTPs) established under *Evaluating Household Water Treatment Options: Health-based targets and microbiological performance specifications* (2011).

Active agent: A substance or medium added to or involved in a drinking water treatment process that requires direct or sacrificial release of the agent or its degradation product(s) to perform a specific functions.

Additive: A substance added to water, directly or indirectly, during a drinking water treatment process.

Backwash: A reversed flow of water through a media which allows the expelling of collected matter to the drain.

Back flush: The references of flow direction through a filter or ion exchange column or membrane to remove particles for cleaning purposes.

Bacteriostatic: A biological or chemical agent that stops bacteria from reproducing, while not necessarily harming them otherwise.

Batch treatment: A method in which a fixed quantity of water is processed through a treatment device in a single treatment cycle.

Capacity: The volume of water treated by a system before the system or components of the system must be cleaned, regenerated or replaced, as specified by the manufacturer.

Challenge water: The mixture of water and contaminants used to test a system for contaminant reduction claims.

Chemical Abstract Service (CAS) Registration Number (RN): Unique numerical identifiers assigned by the Chemical Abstracts Service to every chemical described in the open scientific literature (currently including those described from at least 1957 through the present) and including elements, isotopes, organic and inorganic compounds, ions, organometallics, metals, nonstructurable materials. They are referred to as CAS RNs and CAS Numbers. A CAS RN designates only one substance, has no chemical significance, and provides a link to information about a specific chemical substance. Chemical compounds can be described in many different ways such as molecular formula, structure diagram, systematic names, generic names, proprietary or trade names, or trivial names. A CAS Registry Number, however, is unique and specific to only one substance. CAS Registry Numbers allow for keeping track of substances because they are unique, can be validated quickly and reliably, and are internationally recognized. As CAS RNs are not dependent upon any system of chemical nomenclature, they can provide a reliable common link between the various nomenclature terms used to describe substances and serve as an international resource for chemical substance identifiers used by scientists, industry and regulatory bodies. The assigning agency, Chemical Abstracts Service (CAS) is a function of the American Chemical Society (ACS) and CAS information is copyrighted by the ACS. www.cas.org

Cleaning: Removal of residues and other soiling materials.

Component: A separate or distinct part of a water treatment system including, but not limited to membranes, filters, housings, tubing, storage tanks, faucets, valves, and connectors.

Oo/cyst: The environmentally resistant stage in the life cycle of certain parasitic protozoa which are identified from water samples. These include oocysts of *Cryptosporidium* and *Toxoplasma* and cysts of *Giardia* and *Entamoeba*.

Daily production rate: The volume of product water produced by the system per day under defined conditions.

Disinfection: The process that eliminates (removing, destroying, and inactivating) many or all pathogenic microorganisms with the exception of the bacterial endospore on inanimate objects and liquids.

Effluent: The treated water from the outlet of a unit, system, component, or process.

Filter: (verb) To pass water through a permeable medium to separate particles from the water. (noun) A device for carrying out the process of filtration consisting of the medium and suitable hardware for constraining and supporting the medium in the path of the water.

Filtration: The process by which particles are separated from water by passing water through a permeable material.

Hardness: A measurement of the concentration of divalent and trivalent cations, primarily calcium and magnesium, in drinking water. Hardness is typically expressed as grains per gallon or mg/L as calcium carbonate.

Household Water Treatment (HWT) Technology: A product that is used in households or similar settings to remove water contaminants that may pose health risks. Priority products for testing will be low-cost, appropriate for the poor and generally "free standing" products which only treat enough water to serve a limited number of individuals.

Influent challenge: The mixture of water and contaminants entering a water treatment system.

In-line device: Any device in contact with the water installed on a service line or distribution system downstream of the water main and upstream from endpoint devices.

Media: Material in a system that forms a water-permeable barrier to the passage of certain contaminants or otherwise contributes to the reduction of contaminants in water. Medium is the singular form of media.

Membrane: A semi-permeable barrier that allows the passage of water, and depending on membrane type and characteristics, may restrict the passage of microorganisms, particles, molecules, and ions.

pH: The negative log of the hydrogen ion concentration a measure of the degree of acidity or alkalinity of an aqueous solution.

Post-treatment: The treated water from the outlet of a unit, system, component, or process.

Pretreatment challenge: The mixture of water and contaminants entering a water treatment system.

Pressure: The force applied to a unit area. Water pressure is normally measured in lb/in^2, kilopascals (kPa), or feet or metres of head.

Product water: Water that has been treated by the system.

Rated service cycle: The capacity or time of operation of a system or component between cleaning, replacement, or regeneration of the treatment medium (media), as specified by the manufacturer.

System: A complete water treatment device, including all components needed to connect it to a potable water supply.

Total dissolved solids (TDS): The solids remaining when a solution is filtered through a 0.45 **µ**m

glass filter and the filtrate is evaporated and dried to constant weight at 180 ºC (356 ºF). TDS is expressed as mg solids per litre of filtrate.

Turbidity: A condition caused by the presence of suspended matter, colloidal matter, or both, which results in the scattering and absorption of light.

Unit void volume: Total water-holding volume with the medium (media) and internal components in place.

Unit volume: Total water-holding volume without the medium (media) or internal components.

6. REFERENCES

The following references have been assembled as a single list to cover all Generic Test Plans (GTPs) established under *Evaluating Household Water Treatment Options: Health-based targets and microbiological performance specifications* (2011). As such, not all references are applicable to the GTP of this document.

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- 4500-H^+ pH Value, B. Electrometric Method
- 4500- Cl Chlorine (Residual), G. DPD Colorimetric Method
- 2550-Temperature
- 2540-Solids, C. Total Dissolved Solids Dried at 180^{o}C
- 2320-Alkalinity, B. Titration Method
- 5310-Total Organic Carbon (TOC), C. Persulfate-Ultraviolet or Heated-Persulfate Oxidation Method
- 5310-Total Organic Carbon (TOC), B. High-Temperature Combustion Method
- 9222-Membrane Filter Technique for Members of the Coliform Group**,** B. Standard Total Coliform Membrane Filter Procedure

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