World Health Organization

WHO International Scheme to Evaluate Household Water Treatment Technologies

Harmonized Testing Protocol: Technology Non-Specific

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:

Chemical addition products:

Physical description of the product (liquid, tablet, powder, etc.)

Dissolution time, if applicable

Use pattern or treatment batch volume (Example: 1 tablet/3L)

Required contact time (wait period prior to consumption)

Chemical makeup of the product and the expected residual in the finished product

Shelf life

Batch systems (static treatment), without chemical addition, products:

Use pattern or treatment batch volume

Information on how '*treatment complete*' indicator works

Resource requirements, if applicable

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, if available

Flowing systems (in-line to supplied feed or 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 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

Products with utilize solar and/or thermal technology must include an indicator that alerts the user to when the treatment is complete.

3.1.1. Flowing systems

For flowing systems, three (3) production units shall be selected and run as triplicates (3) in two (2) test waters, except iodine technologies, which shall have four (4) test waters. See iodine specifics below. For systems that include procedures to maintain or restore flow, such as backwashing, the manufacturer shall provide three (3) additional units which have been manufacturer conditioned to stated capacity, including cleaning procedures. These additional pre-conditioned units shall undergo a single microbiological challenge in a single test water.

3.1.2. Chemical addition products

For chemical addition products, three (3) production lots shall be selected and run as triplicates for each lot (3) in two (2) test waters, except iodine products, which shall have four (4) test waters. If the product is manufactured as a continuous process and 'lots' are not appropriate, testing shall use a total of nine (9) replicates of the continuous process product. See iodine specifics below.

3.1.3. Batch systems, without chemical, addition products

Three (3) production lots shall be selected and run as triplicates (3) in two (2) test waters, except iodine products, which shall have four (4) test waters. If the product is manufactured as a continuous process and 'lots' are not appropriate, testing shall use a total of nine (9) replicates of the continuous process product. See iodine specifics below.

Iodine shall require an additional, elevated, temperature during the General Test Water phase to evaluate potential concern for unacceptable levels of iodine in the finished water. Additionally, iodine products shall experience two pH levels during the Challenge Test Water phase. Iodine products will have the same number of overall microbiological data points as non-iodine products. Refer to Microbiological Sample Points.

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. Two (2) types of test water will be used; a general test water (GTW) representing high quality groundwater or rainwater and a challenge test water (CTW) with more aggressive water specifications to representing surface-water. The GTW is not technology specific, and is the same for all products. The CTW, however, is based on the product's technology. Tables 1 and 2 provide the required test water characteristics and adjustment materials for all technologies. 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.

3.2.4. General test water

The GTW is the same for all technologies as it is it not technology specific. 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 (Not technology Specific)

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

3.2.5. Challenge Test Water

The CTW is technology specific and is intended for the stressed challenge phase of testing. Reverse osmosis treated water shall be used as the base water and adjusted to meet the following characteristics:

Table 2: Challenge Test Water Characteristics (Technology Specific)

Constituent	Specification	Adjustment Materials (CAS #[2])
Chlorine[1] (mg/L)	< 0.05	None
pH	Technology dependent	Inorganic acid or base: Hydrochloric acid (7647-01-0) Sodium hydroxide (1310-73-2)
TOC (mg/L)[3]	15 ± 5 mg/L	Humic acid (6813-04-4, Supplier: Alfa Aesar)
Turbidity (NTU)[3]	40 ± 10 NTU	ISO spec. 12103-A2 fine test dust
Temp (°C)	Technology dependent	Not applicable
TDS (mg/L)	1500 ± 150 mg/L	Sea Salts, Sigma Chemical Company (7732-18-5)
Alkalinity[4] (mg/L as $CaCO_3$)	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 measured prior to addition of test water adjustment materials) 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 sea salts.
[2]Chemical Abstract Service registration number. Refer to the definitions of this document for additional information.
[3]TOC and Turbidity added only at microbiological challenge points, except during a 'clogging point' during which all test water may have elevated TOC and turbidity, depending on the product specific test plan.
[4] Intended to buffer pH. Analyzed values may deviate from this range.

3.3. Microbiological Organisms and Challenge Concentrations

Table 3 shows the organisms and American Type Culture Collection numbers (ATCC) used in evaluating performance for all technologies. The target pretreatment concentrations of the organisms for all technologies shall be sufficient to demonstrate: *highly protective, protective, or limited protection.*

Table 3: Microbiological groups and reduction requirements

Microbial group	Pretreatment Challenge[1]	Minimum Required Reduction (log)	
		Highly Protective	*Protective or Limited Protection*
Bacteria: *E. coli* (ATCC 11229)	$\geq 10^5$/100 mL	$\geq$ 4	$\geq$ 2
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 coliphage (ATCC 13706-B1) with host organisms: *E. coli* (ATCC 13706 or ATCC 700078)	$\geq 10^8$/L	$\geq$ 5	$\geq$ 3
Protozoa[4]: *Cryptosporidium parvum* infectious oocysts	$\geq 5x10^5$/L	$\geq$ 4	$\geq$ 2

[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.
[4] Method of analysis (infectivity or total count) shall be dependent on technology of product under evaluations. Challenge oocysts should not be inactivated when evaluating physical removal processes since that changes oocysts flexibility and results in overstated removal performance of mechanical filtration technologies.

3.3.1. Selection of Microorganisms

It is not practical, and there are insufficient data, to set performance targets for all potentially waterborne pathogens. Therefore, the most sensible approach is to identify reference pathogens that represent groups of pathogens. The Scheme reference target organisms were chosen to represent classes of pathogens in water (bacteria, virus and protozoa) with respect to occurrence, concentration and health impact.

For actual testing of performance, selection of microorganisms that represent the three classes of pathogens is necessary. Ideally, surrogates would be chosen for all classes as they are easier and cheaper to use, two important considerations for making the protocol accessible to range of laboratories. However, at this time, there is insufficient data to support selecting surrogates for all classes of pathogens. Thus, the microorganisms selected for inclusion for the HWT Scheme are well documented as laboratory test organisms; they have varying degrees of susceptibility to commonly used drinking water disinfectants; and represent an array of particle sizes/surface properties that should provide useful information with respect to HWTs that rely on mechanical size exclusion for the reduction of microbes.

3.3.1.1. Enteric Bacteria

Enteric bacteria are generally the group of pathogens most sensitive to inactivation by

disinfection. The bacteria species *Escherichia coli* (*E. coli*) shall be used to represent the challenge of bacterial contaminants. *E. coli*, as well as members of the *Enterobacteriaceae* family, has a history of use in disinfection studies and protocols. *E. coli* is typical of the total coliform bacteria group frequently found in untreated surface waters and has added health significance as its presence is very indicative of fecal contamination. Some strains of *E. coli* produce toxin(s) that can lead to severe gastrointestinal illness. According to a recent global study in over 20,000 children in seven developing countries, *E coli* was among the top three pathogens associated with moderate to severe diarrhoea (Kotloff, et al., 2013).

3.3.1.2. 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). The low isoelectric point makes it less sticky (i.e. 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.

3.3.1.3. Parasitic Protozoa

The oo/cysts of parasitic protozoa are the group of pathogens least sensitive to inactivation by chemical disinfection, but relatively sensitive to UV light irradiation, as seen with oocysts of *Cryptosporidium*, which are highly resistant to oxidizing disinfectants such as chlorine. Protozoan oo/cysts are of a moderate size (>2um) and are more readily removed by physical processes compared to viruses and bacteria. Causing the disease Cryptosporidiosis, a severe gastrointestinal illness, *Cryptosporidium hominis* and *C. parvum* are pathogens of concern worldwide and key waterborne reference pathogens cited in the GDWQ (WHO, 2011). According to the same recent, aforementioned study, *Cryptosporidium* is one of the top three

pathogens responsible for diarrhoea in young children in developing countries (Kotloff, et al., 2013). In the environment, the organism exists in a protective cyst stage called an oocyst. *Cryptosporidium* oocysts are typically 3-5 microns in diameter, making it a suitable representative to challenge filtration technologies. *Cryptosporidium parvum* infectious oocysts shall represent the challenge to evaluation protozoa reduction and/or inactivation performance.

For all testing, a total of 1.4L of product water shall be collected and sub-sampled based on analysis sample volume requirements for each microbiological test organism. The 1.4L is sufficient sample size to allow for organism analysis and a retain volume.

3.3.1.4. Organism methods

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

- ***E. coli*** (ATCC 11229) shall be prepared using the method specified in Asburg, E.D. Methods of Testing Sanitizers and Bacteriostatic Substances; in *Disinfection, Sterilization, and Preservation* (Seymour S. Block, ed.) (1983). The samples shall be assayed in triplicate with m-Endo medium using Method 9222B in Standard Methods for the Examination of Water and Wastewater (APHA, 2012). The geometric mean and standard deviation of the triplicate assay shall be reported for each water type and across all water types examined.

    - Collected samples shall be stored at a temperature between 1 – 8°C and processed within 24 hours.
    - Required sample volume to allow for processing in triplicate and a retain volume: 660 ml

- **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: 12ml

- ***Cryptosporidium parvum*** infectious oocysts shall be assayed using an infectivity method which shall be based on a "Most-Probable-Number Assay (MPN) for Enumeration of Infectious *Cryptosporidium parvum* Oocysts", including the standard deviation, as per Slifko *et al*. (1999) for each water type and across all water types examined.

  - Samples shall be stored at a temperature between 1 – 8°C and concentrated by centrifugation within 24 hours of collection.
  - Required sample volume to allow for processing in triplicate and a retain volume: 600ml

- ***Cryptosporidium parvum*** oocysts shall be enumerated using

  - The test method in Annex A of NSF/ANSI 53: Drinking Water Treatment Units – Health Effects (2014); or
  - ISO 15553 Water quality - Isolation and identification of *Cryptosporidium* oöcysts and *Giardia* cysts in water.
  - These methods may be used when a system employs physical removal to reduce *Cryptosporidium parvum*.

    - Collected samples shall be stored at a temperature between 1 – 8°C and processed, stained and mounted within 24 hours.

    - Required sample volume to allow for processing in triplicate and a retain volume: 600ml.

3.4. Other Test Details

3.4.1. Untreated control

The microbiologically spiked test water to be used as the pretreatment/influent challenge concentration, shall also serve as the untreated control. See Table 3 for concentrations. A pretreatment/influent sample shall be collected and split into two samples. One sample shall be neutralized and one shall not be neutralized; these shall determine whether neutralization is not toxic to the microorganisms. The tolerance, between the two samples, must be comparable with intra laboratory reproducibility, which will be specified by the microorganisms and the methods. The neutralized pretreatment/influent sample shall be used in the determination of log reduction.

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. For chemical products the sample will be added to the appropriate treatment volume and sampled for the test organisms of Table 3. 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 3.

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 disinfect residual or wetted contact material of concern

For products that employ a disinfectant, bacteriostatic agent or have a wetted contact material which may have a contaminant leach concern, one product residual sample shall be collected with the microbiology samples from each lot of the post-treatment/effluent samples or from the effluents at each microbiological challenge point. 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. Neutralization

For products that employ a disinfectant, verification of the efficacy of neutralization of the product residual shall be verified for both test waters (GTW and CTW). The Untreated Control shall address potential issues of toxicity of the neutralizer. Common technologies neutralization shall be accomplished through:

- Chlorine shall be neutralized using sodium thiosulfate.
- Iodine shall be neutralized using sodium thiosulfate.
- Silver shall be neutralized using sodium thiosulfate and sodium thioglycolate
- Copper shall be neutralized using sodium thiosulfate and sodium thioglycolate with the addition of lecithin and Tween.

The methods are described in ASTM E1054-08 (2013)

3.4.6. Microbiological sample points

The microbiological addition to the test water and post treatment/effluent sample collection points are determined by the operation of the product.

3.4.6.1. Chemical addition products

For chemical addition (batch) systems, three (3) production lots shall be selected and run as triplicates (3) per lot in two (2) test waters for each test organism, except for iodine products which shall have four (4) test waters. Use instruction on wait or mixing times shall be used in testing. If the product instruction specifies a type of container material, this shall be used for the testing. However, it the manufacturer does not specify in their product literature, the most conservative test container material shall be used, which typically would be glass. Glass is expected to have more adsorption to the container walls and therefore would be considered to be most conservative. To be certain there is no carryover adsorption from previous product exposure; all chemical products shall be tested using new test vessels each time.

Non-iodine products

Sample Collection for GTW:

- 1 blank sample analyzed for the organism of Table 3
- 1 sample for Test Water Characteristics
- 1 pretreatment sample, neutralized and analyzed for organism of Table 3
- 1 pretreatment sample (not neutralized) analyzed for organism of Table 3
- 9 post-treatment samples analyzed for organism of Table 3
- 3 post-treatment samples for product residual (one per triplicate run)

Sample Collection for CTW:

- 1 sample for Test Water Characteristics

- 1 pretreatment sample, neutralized and analyzed for organism of Table 3
- 1 pretreatment sample (not neutralized) analyzed for organism of Table 3
- 9 post-treatment samples analyzed for organism of Table 3
- 3 post-treatment samples for product residual (one per triplicate run)

Iodine products

Sample Collection for GTW (20 ± 3°C):

- 1 blank sample analyzed for the organism of Table 3
- 1 pretreatment sample, neutralized and analyzed for organism of Table 3
- 1 pretreatment sample (not neutralized) analyzed for organism of Table 3
- 6 treated samples analyzed for organism of Table 3
- 3 treated samples for product residual (one per triplicate run)

Sample Collection for GTW (35 ± 2°C):

- 1 pretreatment sample, neutralized and analyzed for organism of Table 3
- 1 pretreatment sample (not neutralized) analyzed for organism of Table 3
- 3 treated samples analyzed for organism of Table 3
- 3 treated samples for product residual (one per triplicate run)

Sample Collection for CTW pH 9.0:

- 1 pretreatment sample, neutralized and analyzed for organism of Table 3
- 1 pretreatment sample (not neutralized) analyzed for organism of Table 3
- 6 treated samples analyzed for organism of Table 3
- 3 treated samples for product residual (one per triplicate run)

Sample Collection for CTW pH 5.0:

- 1 pretreatment sample, neutralized and analyzed for organism of Table 3
- 1 pretreatment sample (not neutralized) analyzed for organism of Table 3
- 3 treated samples analyzed for organism of Table 3
- 3 treated samples for product residual (one per triplicate run)

3.4.6.1.1. Dose based on 25th percentile of drop size

For products which are administered via dropper, the following procedure shall be used: Three (3) technicians, each using a different manufacturer provided dropper, shall each deliver and weigh 20 drops of the product on a calibrated analytical scale. All weights shall be recorded and the 25th percentile of the total drops identified. The 25th percentile volume shall be the volume used, delivered via calibrated pipette, during the testing.

3.4.6.2. Batch systems without chemical addition products

Testing shall be based on the product '*treatment complete*' indicator, unless there is proper justification to approach differently. For example, if a solar product's indicator is activated by heat only, testing shall provide the system with heat only to evaluate performance at the indication of the treatment being complete. The test plan shall include a sample schedule which will require consideration of the time required for a batch treatment and the number batches that can be treated in a single day. Sampling of microbiological organisms shall occur at the time that '*treatment complete*' is indicated.

Sample Collection for GTW:

- 1 blank sample analyzed for the organism of Table 3
- 3 pretreatment sample analyzed for organism of Table 3
- 1 sample for Test Water Characteristics
- 9 post-treatment samples analyzed for organism of Table 3

Sample Collection for CTW:

- 1 blank sample analyzed for the organism of Table 3
- 3 pretreatment sample analyzed for organism of Table 3
- 1 sample for Test Water Characteristics
- 9 post-treatment samples analyzed for organism of Table 3

3.4.6.3. Flowing systems

Sampling for microbiological organisms shall be conducted according to a sample schedule, which is based on the days on test and the technology specified microbiological sample days. The test duration shall be based on technology resulting in testing to the capacity or until 'clogging' for systems that clog with use.

- For system that experience reduced flow (clogging) with use, the sample schedule shall include instruction on end of test if the system's flow or other indicator of 'end of life' has not been reached by Day 9. Instruction will be identified in the product specific test plan for accelerated clogging by the addition of the Table 2 specification for TOC and turbidity during all test water, not just the microbiologically challenged water following Day 9. This shall be referred to as a 'clogging point' sample.
- Additionally, for systems with procedures/capabilities to maintain or restore flow, such as backwashing, three (3) additional units which have been conditioned by the manufacturer to the manufacturer stated capacity, including cleaning procedures shall undergo a single microbiological challenge in General Test Water for the two most rigorous classes of organisms for the technology.
  - For example, a filtration product shall have a single microbiological challenge point for bacteria and virus.
  - If a product is seeking *limited protection*, the two classes identified for the full test shall be used for the pre-conditioned units.

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 3 immediately prior to sample collection and continued through sample collection. For batch flowing systems, a full batch may be used for seeding and a full batch shall be 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 3
- 3 pretreatment sample analyzed for organism of Table 3
- 1 sample for Test Water Characteristics
- 9 post-treatment samples analyzed for organism of Table 3
- For systems requiring pre-conditioned unit to be tested:

- 1 sample for Test Water Characteristics (GTW)
- 1 pretreatment sample analyzed for organism of Table 3
- 3 post-treatment samples analyzed for organism of Table 3

Sample Collection for CTW:

- 1 blank sample analyzed for the organism of Table 3
- 3 pretreatment sample analyzed for organism of Table 3
- 1 sample for Test Water Characteristics
- 9 post-treatment samples analyzed for organism of Table 3

Flowing systems with chemical disinfection shall require neutralization and residual disinfection concentration analysis as discussed under Section 3.4.6.1 Chemical addition products.

3.4.7. Conditioning

For systems that require conditioning, conditioning shall be according to the Operation Manual. The general test plan for the product type provides direction on conditioning prior to testing. Conditioning shall use GTW for short term conditioning (single day) and de-chlorinated tap water for long conditioning (greater than a single day). 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.

3.4.8. Cycling

Cycling is the starting and stopping of flow as would occur in actual use. Cycling may be appropriate for flowing systems, particularly those plumbed in-line to piped water supplies. For batch systems, cycling shall coincide with batch processing.

3.4.9. End of life

For flowing and batch systems, the manufacturer must provide an explicit indication or assurance of the unit's effective use lifetime to warn the consumer of potential diminished treatment capacity by one of the following:

- Having the unit terminate discharge of treated water
- Sounding an alarm
- Providing single explicit instructions for servicing or replacing units within the recommended use life (measureable in terms of volume throughput, specific timeframe or other appropriate method).

3.4.10. Daily test capacity

For flowing systems, the Operator's Manual may supply the daily capacity of the system and the system shall be run accordingly, but not to exceed 8 total hours of system flowing in a single test day. For batch systems and chemical addition products, daily test capacity will be based on product use, time for treatment and laboratory efficiency.

3.4.11. Leakage test

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

3.4.12. Device cleaning

For systems, approaches to restore or maintain flow identified in the Operator Manual shall be

permitted during testing. The general test plan for the product type provides direction on device cleaning during testing.

3.4.13. Component replacement

For systems, a component that would not be considered a primary component in providing the microbiological reduction performance may be replaced as needed during the test. An example is a pre-filter for turbidity removal. However, a component which provides microbiological performance shall not be replaced during the testing. The general test plan for the specific technology provides direction on component replacement during testing.

3.4.14. End of test

The general test plan for the product type shall provide clear direction on 'end of test'. For chemical addition products, end of test shall be completion of the test plan and collection of all data. For flowing system devices, there shall be two (2) acceptable outcomes for the end of the test: completion of the Sampling Schedule, which includes at least 9 full days of flowing followed by an accelerated clogging point initiated on Day 10 or clogging during the sample schedule prior to the final collection point.

3.4.15. Log reduction calculation

Testing shall be conducted simultaneously on the technology 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:

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

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:

$$\text{Log}_{10}\ 2.07\text{+E8} - \text{Log}_{10}\ 8.95\text{E+01} = \text{log reduction}$$
$$8.31 - 1.95 = 6.36 \text{ log reduction}$$

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 3, except for the following acceptable allowance. Up to 10 % of pretreatment/post-treatment sample pairs may vary from the reductions required in Table 3 but not achieve less than:

Viruses:	1 log variance
Bacteria:	1 log variance
Oocysts:	1 log variance

Each phage is treated separately for evaluating acceptable allowance, however the overall claim for virus shall 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 3. 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

Exact and detailed testing procedures for chemical addition or flowing system devices shall be put in the product specific test plan developed for each product to be evaluated by the testing laboratory.

For all testing, however, test waters shall be prepared daily and verified in accordance with Tables 1 and 2. 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.

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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