World Health Organization

WHO International Scheme to Evaluate Household Water Treatment Technologies

General Testing Protocol #1: Chlorine Chemical Point of Use Disinfectants Technology

Geneva, Switzerland

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Table of Contents

List of Tables

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

Designated Test Laboratory

The designated testing laboratory shall be identified.

2. PURPOSE

The household water treatment (HWT) product that are direct addition (non-system) chlorine disinfection 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

Three (3) production lots shall be selected and run as triplicates for each lot (3) in two (2) 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.

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 represent surface-water. Tables 1 and 2 provide the required test water characteristics and adjustment materials for chlorine disinfection 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.

3.2.1. 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.

3.2.2. Challenge Test Water

The CTW 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

Constituent	Specification	Adjustment Materials (CAS #[2])
Chlorine[1] (mg/L)	< 0.05	None
pH	9.0 ± 0.2	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)	4 ± 1°C	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 chlorine chemical disinfection products. The target pretreatment concentrations of the organisms shall be sufficient to demonstrate: *highly protective, protective, or limited protection.*

Table 3: Microbiological organisms and reduction requirements

Organism	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 coliophage (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: 660ml
- **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

3.4. Other Test Details

3.4.1. Untreated control

The microorganisms spiked test water to be used as the pretreatment/influent challenge concentration for testing, 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 sample 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.

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

One product residual sample shall be collected with the microbiology samples from each lot of the post-treatment/effluent samples. 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

Verification of the efficacy of neutralization of the product residual shall be confirmed for both test waters (GTW and CTW). The Untreated Control shall address potential issues of toxicity of the

neutralizer. Chlorine shall be neutralized using sodium thiosulfate The methods are described in ASTM E1054-08 (2013)

3.4.6. Microbiological sample points

Three (3) production lots shall be selected and run as triplicates (3) per lot in two (2) test waters for each test organism. Manufacturer provided use instruction on wait and/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.

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)

3.4.6.1 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.

3.4.7. Daily test capacity

For batch systems and chemical addition products, daily test capacity will be based on product use, time for treatment and laboratory efficiency.

3.4.8. Component replacement

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.

3.4.9. End of test

For chemical addition products, end of test shall be completion of the test plan and collection of all data. The general test plan for the product type shall provide clear direction on 'end of test'. In the event that a chlorine disinfection chemical addition product included an indicator of water treatment 'complete', there shall be direction in the test plan for 'end of test' should the indicator not signal completion of treatment.

3.4.10. 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:

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

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:

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

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

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

For all testing, 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.

1. Three (3) production lots shall be selected and run in triplicate (3) in two (2) test waters for each test organism.
2. Test waters shall be prepared daily and verified in accordance with Tables 1 and 2.
    a. Testing in each test water (GTW and CTW) may each be run as separate events, however, all replicates of a single test water type must be run simultaneously.
    b. Daily test water characteristics shall be sampled, analyzed and results provided in the final report. Sufficient volume to allow for a sample retain shall be collected. One shall be used for analysis and reporting. The backup volume shall be retained for confirmation or retesting purposes, when necessary.
3. Microbiologically spiked challenge water shall be prepared to meet the concentrations of Table 3. All organisms (*E. coli*, MS-2, PhiX-174 and *Cryptosporidium parvum* oocysts) are compatible for combined challenge.
4. The product shall be prepped and employed according the use instructions of the product instruction manual.
5. Test water that has been microbiologically spiked according to Table 3 shall be dosed with product according the use instructions of the product literature.
    a. Per the manufacturer's instruction for use, the product shall be added to the manufacturer indicated volume for three (3) lots, with each lot run in triplicate (3).
    b. After the addition of the product to the test water, the test contact/wait time shall begin (t=0).
    c. If agitation is indicated in the use instructions, the test vessel may be set on a rocker or shaking platform set to a setting that is consistent with the use instructions or may be agitated manually by the technician. Instructions may also dictate inversion which can be accomplished with test vessels that have stoppers.
    d. Laboratory technician shall record any observations of interest relative to the product dissolution, color, characteristics variation by lot, etc. in the laboratory bench sheets.
6. The microbiological post-treatment and disinfectant residual (active agent) samples shall be collected after completing the manufacturer dictated mixing and/or wait instructions.
    a. Samples shall be neutralized immediately upon collection.
    b. Sufficient volume to allow for a retain volume shall be collected. One sample volume shall be used for analysis and reporting. The backup volume shall be retained for confirmation or retesting purposes, when necessary.
7. Pretreatment challenge and disinfect residual (active agent) samples shall be collected from the microbiologically spiked challenge water and analyzed to confirm pretreatment concentrations.
    a. As the pretreatment challenge water for each set of triplicates was from a single source of microbiologically spiked prepped test water, a single pretreatment sample per triplicate shall be taken and for analyzed for the organisms of Table 3.
    b. The pretreatment microbiological shall be collected immediately from the microbiologically spiked test water after all post-treatment samples have been collected.

c. Sufficient volume to allow for a retain volume shall be collected. One sample volume shall be used for analysis and reporting. The backup volume shall be retained for confirmation or retesting purposes, when necessary.

8. Microbiological pretreatment and post-treatment 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 WHO International Scheme to Evaluate Household Water Treatment Technologies.

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- Cl Chlorine (Residual), G. DPD Colorimetric Method
- 2550-Temperature
- 2540-Solids, C. Total Dissolved Solids Dried at 180°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
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