World Health Organization

WHO International Scheme to Evaluate Household Water Treatment Technologies

Harmonized Testing Protocol: Technology Non-Specific
Version 2.1

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Glossary

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 technology-specific test plans 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 Test Water (CTW): Laboratory created test water that uses identified adjustment materials to simulate surface water.

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.

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 treated 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.

General Test Water (GTW): Laboratory created test water that uses identified adjustment materials to simulate high quality ground water or rainwater.

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) product: 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 settings without reliable piped water or power and generally "free standing" products which only treat enough water to serve a limited number of individuals.

Household water treatment (HWT) technology: The method or process by which household water treatment products remove microbiological contaminants from drinking-water. Physical methods include boiling, heating (using fuel and solar), filtering, settling and ultraviolet (UV) radiation (solar or UV lamps). Chemical methods include coagulation–flocculation and precipitation, ion exchange, chemical disinfection with germicidal agents (primarily chlorine) and adsorption.

Influent challenge: The mixture of water and contaminants entering a water treatment system. Synonymous with "pre-treatment challenge".

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. Synonymous with "effluent".

Pre-treatment 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.

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 180ºC (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.

1 UPDATES FROM PREVIOUS PROTOCOLS V1.0 AND V2.0

The objective of this Harmonized Testing Protocol is to evaluate household water treatment (HWT) products for microbiological reduction and/or inactivation performance based on recommendations and testing principles set forth in the World Health Organization's (WHO) *Evaluating Household Water Treatment Options: Health-based targets and microbiological performance specifications* (2011). Testing conducted by 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 (the Scheme) Procedure for Evaluation (Procedure).

The aim of the protocol is to guide designated testing laboratories, as well as other laboratories interested in testing according to WHO recommendations, in evaluating HWT products in the most scientifically rigorous as well as most efficient and cost effective way possible. The latter is especially important to ensure that all HWT products of public health relevance are evaluated and in the future to allow for more testing in low- and middle-income countries where the majority of these products are distributed. Based on the aforementioned, several changes from the Harmonized Protocol Version 1.0 and 2.0 have been made to the current 2.1 version. These changes are outlined below.

Methods (Section 4)

Reduction in the number of total samples (Replicate samples, Section 4.1; Flowing systems, Section 4.1.1; Chemical disinfectants Section 4.1.2 and Batch systems without chemical addition 4.1.3)

For chemical disinfectants, the total number of samples has been reduced from 18 to 12. Specifically, the production lots have been reduced from three to two production lots and there has been a reduction from nine to six replicates for chemicals manufactured as a continuous process. Under the Scheme evaluation Procedure, manufacturers must present evidence of utilizing the principles of ISO 9001:2015 Quality Management Systems (or an equivalent) as a means to demonstrate quality assurance of manufacturing and consistency in production.

For flowing systems, including batch treatment, the total number of sample collection points has been reduced from six to four. As devices are tested in triplicate, the resulting number of post treatment samples is reduced from 18 to 12. Unless the product use instructions would imply a different schedule, the sampling shall occur at: start (post-conditioning, if applicable); end of the General Test Water (GTW) phase; change to Challenge Test Water (CTW) phase; and final collection during CTW. The sampling points identified are to be the most challenging for the technology, thus providing a rigorous evaluation of performance.

The sampling points were reduced based on the data from the ten products tested in Round I which found that the number of testing points were greater than necessary to determine performance and added time and cost to the testing.

Chemical disinfectants (Section 4.1.2)

A disinfectant pre-check shall now be performed on all disinfectant technologies prior to proceeding to the full performance evaluation for microbiological inactivation to confirm product is provided disinfectant in the range indicated by the manufacture and to confirm consistency in manufacturing.

Test waters (Section 4.2)

Chemical disinfectants (as direct additives or chemical generator devices)

- Chemical demand (as chlorine) shall be added as a test water specification
- Total Organic Carbon (TOC) shall no longer be a test water specification. Tannic and humic acid shall be used to adjust the chemical demand of the test waters and the volume of the WHO

Scheme identified TOC stock solutions shall be provided for guideline additions (see Section 4.2.2)

- Due to the variability by lot of naturally sourced humic acid, tannic acid shall be used as the TOC adjustment material for both the general test water and the challenge test water.

Ultra-violet (UV) disinfection technologies (no specific section to reference)

For UV technologies, the CTW characteristics will be based on whether the system has a performance indicator with the intention of evaluating the performance just below the point of alarm. Refer to section 4.2 Tables 1 and 2 for the the use of Parahydroxybenzoic Acid (PHBA) to adjust the test water specification of color U.V. Absorption 254nm, where appropriate.

Microbiological organisms and challenge concentrations (Section 4.3)

Bacteria pre-treatment challenge concentration

The pre-treatment challenge concentration for bacteria is a higher concentration than that stated in Version 1.0 of the Harmonized Testing Protocol and greater than that needed to allow for the demonstration of the required log reduction under the Scheme, allowing for the evaluation of performance of up to 6 log. The performance data resulting from the higher pre-treatment target concentration may prove to be useful as other local protocols known to require higher log reductions for bacteria, such as the United States Environmental Protection Agency (US EPA) Guide Standard and Protocol for Testing Microbiological Water Purifiers (1987). However, the Scheme will only report publicly on whether the criteria of WHO Scheme was met. This data may also prove useful when it is not possible to include other microbial groups in the evaluation and a relationship between *E. coli* and expected performance of other microbial groups can be considered.

Reduction in microbial groups to be evaluated

To the extent that there is convincing and substantial evidence about the performance of technologies against certain microbial groups, it may be possible to reduce the number of microbial groups required for the evaluation. For example, it is well documented that chlorine is ineffective against protozoa, especially *Cryptosporidium*. Thus, for this particular HWT technology, no testing would be done for protozoa and removal would be noted as 0 $\log_{10}$. In all cases, the decision to not require a microbial group(s) is at WHO's discretion with input from the Independent Advisory Committee (IAC). Refer to the technology specific protocol for required microbial groups for the evaluations.

Removal of blank sample

The original intent of testing a blank sample was to identify if a product was contaminated with one of the test organism upon product receipt. Based on the extensive experience of laboratories that test household treatment products and on data from Round I of testing, the likelihood that a product would be contaminated before testing is rare. Thus, the blank sample has been removed. The quality assurance/quality control (QA/AC) requirements of Section 4.4.2 address proper laboratory practice to, among other precautions, avoid contamination at the designated testing laboratory.

Neutralization (Section 4.4.4)

The designated laboratories have extensive experience with the neutralization of disinfectants, particularly for those most commonly used: chlorine, iodine, copper and silver. The product-specific test plan developed by the designated laboratories and reviewed and approved by WHO and the manufacturer shall require the demonstration of complete disinfection and no toxic effect to test organisms. However, rather than using the prescriptive approach dictated in the Version 1.0 of the Harmonized Test Protocol (American Society for Testing and Materials (ASTM) E1054-08 (2013)), laboratories may conduct neutralization in a more targeted approach that may require less samples. Therefore the specific reference to neutralization as a requirement has been removed.

Interpretation of results (Section 6)

Change to the performance classification nomenclature

Based on input from a variety of stakeholders, including Member States, HWT manufacturers, international organizations and non-governmental organizations, the performance classification nomenclature has been modified to reflect more objective and direct categorization of HWT performance. Thus performance classification was changed from: *Highly protective*; *Protective* and *Limited protection* to: *Comprehensive protection*: three-star (★★★); *Comprehensive protection*: two-star (★★); and *Targeted protection [microbial groups the product is protective against]*: one-star (★). Section 5 provides detailed information on the criteria for each performance classification.

It is recognized that information is needed to help guide procurers and users in understanding performance along with other important factors influencing correct and consistent use and ultimately health benefits including cost, ease of use and accessibility of spare parts and replacements. Such decisions will require assessing many trade-offs which are often context specific. While such recommendations extend beyond the main normative function of the Scheme, Section 6 has been added to provide some further details on interpretation of the results to better guide the selection of HWT products for a particular setting.

2 PRODUCT INFORMATION REQUIREMENTS

The manufacturer is required to provide detailed product information within the Expression of Interest (EoI)[1] in order to: determine if a product is appropriate for testing; develop the specific test protocols; and conduct the actual testing. This information includes:

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).

[1] Refer to the WHO website for the most recent EOI: http://www.who.int/household_water/scheme/applicant/en/

- Manufacturer capacity and supporting information upon which capacity is based.

3 PURPOSE

The HWT product shall be evaluated for microbiological performance based on recommendations and testing principles set forth in the *Evaluating household water treatment options: Health-based targets and microbiological performance specifications* (WHO, 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 Scheme Procedure for Evaluation.

The Harmonized Testing Protocol is intended to provide an overview of the evaluation criteria and inclusions. For specific information by technology type, refer to the technology specific protocols in all cases.

4 METHOD

4.1 Replicate samples

The replicate samples required for the various treatment technologies are outlined below.

4.1.1 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.

4.1.2 Chemical addition products

For chemical addition products, two (2) production lots shall be selected and run as triplicates (3) for each lot 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 six (6) replicates of the continuous process product. See iodine specifics below.

A disinfectant pre-check shall be performed on all chemical disinfectant technologies prior to proceeding to the full performance evaluation for microbiological inactivation to confirm product is provided disinfectant in the range indicated by the manufacture and to confirm consistency in manufacturing.

4.1.3 Batch treatment systems without chemical addition

Three (3) units representing different production lots (where practical and available) shall be selected and run as triplicates (3) in two (2) test waters, except iodine products, which shall have four (4) test waters.

Iodine shall require an additional, elevated, temperature during the General Test Water (GTW) 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 (CTW) phase. This is necessary because the effectiveness of iodine against microorganisms is highly dependent on temperature and pH. Iodine device products will have the same number of overall microbiological data points as non-iodine products. Iodine chemical products shall require more sample points. Refer to Microbiological sample points (Section 4.4.5).

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

4.2 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 types of test water will be used; a GTW representing high quality groundwater or rainwater, and a CTW with more aggressive water specifications to

representing surface-water. The GTW is not technology specific, and, for most technologies and where possible, is the same for all products. The CTW, however, is based on the product's technology. Tables 1 and 2 provide the typical test water characteristics and adjustment materials for all technologies, however it is important to refer to the technology specific protocol for exact and technology specific specification. 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. When identified as a specification, Total Organic Carbon (TOC) is verified during test water preparation 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
- TOC2: Tannic acid for GTW and humic acid for CTW 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

4.2.1 Total organic carbon (TOC) specification

Tannic acid preparation

Tannic acid addition shall be from a stock solution prepared as: 6 g of tannic acid powder dissolved in 1 L of reverse osmosis (RO) or deionized water (DI). The prepared solution shall be stored in an amber bottle, protected from light and air and held no longer than 7 days.

The single, above described tannic acid stock shall be made from the dry powder; there shall be no intermediate stock solution. The formula may be scaled up or down provided the relative concentrations are maintained.

Humic acid preparation

Humic acid addition shall be from a stock solution prepared as: 6 g of humic acid powder dissolved in 1 L of RO/DI water. Using sodium hydroxide, the solution is to be adjusted to a pH of 9-10 to increase the solubility of the humic acid, reduce the amount of precipitates and allow for increased stability. The prepared solution shall be stored in an amber bottle, protected from light and air and held no longer than 7 days.

The single, above described humic acid stock shall be made from the dry powder; there shall be no intermediate stock solution. The formula may be scaled up or down provided the relative concentrations are maintained.

4.2.2 General Test Water

The General Test Water (GTW) represents the non-stressed phase of testing. Reverse osmosis treated water shall be used as the base water and adjusted to meet the following characteristics presented in Table 1:

2 The two TOC compounds have different characteristics and interactions with different technologies and thus allow for the evaluation under different use conditions and environments, increasing the validity and relevance of the testing. Tannic acid reacts faster with oxidative technologies, is more soluble and less sensitive to the ionic content of the water. Humic acid, with a higher molecular weight, is less stable, may complex with divalent cations present in many waters and typically contains particulate which may assist in the clogging of pores.

Table 1. General Test Water characteristics

Constituent	Specification	Adjustment Materials (CAS#[1])
Chlorine[2] (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)
Chemical demand (as chlorine)[3]	1.5 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[4] (mg/L as CaCO3)	100 ± 20 mg/L	Sodium bicarbonate (144-55-8)
Color U.V. Absorption 254nm[5]	Technology dependent	Measured – no addition

[1] Chemical Abstract Service registration number. Refer to the definition section of this document for additional information.

[2] 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.

[3] Specification and measured and reported only for chemical disinfectants.

[4] Intended to buffer pH. Analyzed values may deviate from this range.

[5] UVT shall be measured by a UV % transmission photometer.

4.2.3 Challenge Test Water

The Challenge Test Water (CTW) is technology-specific and is intended for the stressed challenge phase of testing. For ultra-violet (UV) technologies, the CTW characteristics will be based on whether the system has a performance indicator with the intention of evaluating the performance just below the point of alarm. Refer to the technology specific test plan for testing details for UV and all technologies. Reverse osmosis-treated water shall be used as the base water and adjusted to meet the following characteristics presented in Table 2:

Table 2. Challenge Test Water characteristics

Constituent	Specification	Adjustment Materials (CAS #[1])
Chlorine[2] (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)
Chemical demand (as chlorine) [4]	1.5 mg/L	Tannic acid (1401-55-4, 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[5] (mg/L as $CaCO_3$)	100 ± 20 mg/L	Sodium bicarbonate (144-55-8)
Color U.V. Absorption 254nm[6]	Technology dependent	Parahydroxybenzoic Acid (PHBA)

[1] Chemical Abstract Service registration number. Refer to the definitions of this document for additional information.
[2] 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.
[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] Specification and measured and reported only for chemical disinfectants. Tannic shall be used for chemical disinfectants as it is less variable from lot to lot than humic acid.
[5] Intended to buffer pH. Analyzed values may deviate from this range
[6] Unil alarm activates. No addition may be required if alarm activates by TOC addition. UVT shall be measured by a UV % transmission photometer.

4.3 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 pre-treatment concentrations of the organisms for all technologies shall be sufficient to demonstrate: 3-star (★★★), 2-star (★★), or 1-star (★). Note that the pre-treatment challenge concentration for bacteria is higher than necessary to demonstrate Scheme performance targets.

Table 3. Microbial groups and reduction requirements

Microbial group	Target pre-treatment challenge[1]	Minimum required reduction (log_{10})	
		★★★	★★
Bacteria[2]: *Escherichia coli* (ATCC 11229)	$\geq 10^6$/mL	≥ 4	≥ 2
Virus[3,4]: 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)	$\geq 10^5$/mL	≥ 5	≥ 3
Protozoa[5]: *Cryptosporidium parvum* infectious oocysts	$\geq 5x10^4$/mL	≥ 4	≥ 2

[1] Pre-treatment challenges may constitute greater concentrations than would be anticipated in source waters, but these are necessary to properly test, analyse, and quantitatively determine the indicated log reductions. Pre-treatment challenge must not be less than that required to demonstrate the geometric mean and standard deviation minimum required reduction described below. Pre-treatment concentrations presented in table in harmonized units. Refer to Section 4.3.2 organism methods for actual volumes processed and method sensitivity. Pre-treatment concentrations are intended to allow for the demonstration of: 6 log for bacteria, 5 log for virus and 4 log for protozoa.

[2] Influent target higher than required by the Scheme as demonstration of 6 log_{10} may be required of other schemes.

[3] Virus performance claim will be based on the poorest log reduction of the two phages.

[4] Host selection is dependent on method. Refer to Section 4.3.2 Organism Methods.

[5] 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.

Based on the best available evidence and WHO's discretion, the microbial groups used in the performance evaluation may be reduced. The following are considerations of such technologies:

- *Filters:* The microbial groups and the identified surrogates evaluated under the Scheme represent a range of physical sizes, from the largest being 3-5 microns in diameter for *Cryptosporidium* to the phages such as MS2 and phi-X174 which are approximately 24 nm and 27 nm respectively in diameter. As such, for filters that are based solely on size exclusion it may be acceptable to base the evaluation on the product's reduction performance for the bacteria and virus microbial groups only. Evaluation against protozoa, the largest in size of the microbial groups, would not be required. Ultra-filtration is an example of a candidate technology for this consideration. However, it is important that the mechanism of removal for the technology is well understood. For example, for filters which rely primarily on adsorption, such as carbon, rather than size exclusion, it is necessary to test against all three classes of pathogens.
- *Chemical disinfectants*: Due to their physical characteristics, the protozoan microbial group represents a very rigorous challenge for disinfecting technologies (WHO, 2011). Therefore, unless there is justification (including supporting data) for why a disinfectant product may perform well against the protozoan microbial group (e.g. pre-filtration, etc.), these products will not be tested against protozoa (*Cryptosporidium*) and thus the highest performance level they could achieve is 1-star (★) for reduction of *Escherichia coli* (*E. coli)*, MS2 and phiX-174 only.

- *UV disinfection*: Performance for the protozoan microbial group shall be based on the performance outcome of the bacteria microbial group. Due to *Cryptosporidium*'s sensitivity to UV disinfection, such that 3-4 log reduction in both *Cryptosporidium* (Clancy et al., 2000) and *Giadia* (Craik et al., 2000) may be accomplished by a UV dosage of 10 mJ/cm^2, compared to the somewhat more resistant *E. coli* used to represent the bacterial microbial group can be used as a conservative indicator of expected performance for the protozoa group. Most vegetative bacteria, including coliform species, are susceptible to UV radiation at a dose of 16,000 uW-sec/cm^2. In one study, survival data (Chang et al., 1985) show that a greater than 2 log reduction of the non-spore forming heterotrophic bacteria may be accomplished by a UV dose of 16,000 uW-sec/cm^2.
- *Solar / thermal disinfection:* Thermal inactivation has been examined in water and other liquids at temperatures close to those used for pasteurization (e.g. 63°C for 30 minutes, 72°C for 15 seconds) and in hot water (about 60°C) and been shown to be effective against bacteria, viruses and protozoan cysts. For *Cryptosporidium*, reductions of greater than 3 log have been observed after exposure to temperatures of 60-72°C for 1-5 min (Fayer, 1994), and similar reductions have been observed for a range of bacteria (WHO, 2015). As such, it may be acceptable to base the evaluation on the product's reduction performance for the bacteria and virus microbial groups only.

4.3.1 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 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 especially in low resource settings. However, at this time, there is insufficient data to support selecting surrogates for all classes of pathogens. Thus, the microorganisms selected for inclusion in the 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 HWT technologies that rely on mechanical size exclusion for the reduction of microbes.

4.3.1.1 Enteric bacteria

Enteric bacteria are generally the group of pathogens most sensitive to inactivation by disinfection. The bacteria species *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).

4.3.1.2 Enteric viruses

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 latter option was

chosen. 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, 25-27 nm 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.

4.3.1.3 Parasitic protozoa

The oocysts 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 (>2 um) and are more readily removed by physical processes compared to viruses and bacteria. Causing the disease Cryptosporidiosis, a severe gastrointestinal illness, *Cryptosporidium hominis* and *Cryptosporidium parvum* (*C. parvum*) are pathogens of concern worldwide and key waterborne reference pathogens cited in the GDWQ (WHO, 2017). 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, and thus similar in size to other oocysts. For these reasons, *C. parvum* infectious oocysts shall represent the protozoan class. Cryptosporidium is difficult to obtain (supplies are limited) and requires an intensive process for handling. Thus, as more evidence becomes available on the suitability of using, other less pathogenic microorganism or surrogates including microspheres such alternatives will be pursued.

For all testing, a total of 1.4 L of product water shall be collected and sub-sampled based on analysis sample volume requirements for each microbiological test organism. The 1.4 L is sufficient sample size to allow for organism analysis and a retain volume, should the analysis need to be re-run within hold times for any reason, such as due to lab error, confirmation, etc.

4.3.2 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.

- Organisms in stationary phase of growth and suspended in phosphate buffered saline shall be used. Growth phases shall be determined using optical density (OD) and strain specific standard curves developed by the designated testing facility. The growth phase of the cultures shall be confirmed each time the cultures are prepared.
- 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: 12 ml

- **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 Part 2: 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

- ***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: 600 ml

- ***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* oocysts and *Giardia* cysts in water.
  - These method 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: 600 ml.

4.4 Other test details

4.4.1 Untreated control

The microbiologically spiked test water to be used as the pre-treatment/influent challenge concentration, shall also serve as the untreated control. See Table 3 for concentrations. A pre-treatment/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 has a toxic effect on 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 pre-treatment/influent sample shall be used in the determination of log reduction.

4.4.2 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 and testing environmental controls.

4.4.3 Product disinfectant 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 (2017) shall be used to determine acceptable levels in the treated water.

4.4.4 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 as follows:

- 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.

Based on available literature, the test organism that is most sensitive to the tested product shall be used for the confirmation of neutralization effectiveness and to address toxicity concerns. The following approach shall be used prior to the test for both GTW and CTW to confirm neutralization effectiveness and that the neutralization is not toxic to the test organisms:

Preparation of test solutions

- In a flask (A), prepare 100 mL of test water with the product at testing concentration, to analyze for neutralizer effectiveness.
- In a second flask (B), prepare 100 ml of test water for use in analyzing neutralizer toxicity
- In a third flask (C), prepare 100 mL of test water for use as a quantitative organism viability control.

Note: This will result in a total of 6 flasks: A, B and C for GTW and CTW each

Procedure

- Add the neutralizer to flask A and B at the test concentration and volume; mix thoroughly
- Add sufficient organism to flasks A, B, and C to achieve a final number of +/- 100 CFU or PFU per plate; mix thoroughly.
- Following a minimum 5 minute wait time, transfer sufficient volume from each flask to process on duplicate plates.
- Dilute a sufficient volume from each flask in a 1:1 ratio (2 × dilution) and process duplicate plates.
- The processing method, media, incubation conditions, etc. used should be according to Section 4.3.2 Organism methods.

Note: This will result in 4 plates per flask, 2 each for the diluted and undiluted samples.

Data Analysis

Average the counts for the duplicate replicates from each flask then calculate reduction factors (X) using the following:

- Neutralizer Effectiveness: C/A = X
- Neutralizer Toxicity: C/B = X

The reduction factor (X) shall not be greater than 2 for either test.

- A reduction factor greater than 2 for the Neutralizer Effectiveness test indicates that the neutralizer used was not effective.
- A reduction factor greater than 2 for the Neutralizer Toxicity test indicates that the neutralizer used is toxic to the organism.
- If the reduction factor is greater than 2 for either test, a retest is required utilizing a different neutralization method.

Options for alternate neutralization methods are below and should be chosen based on the outcome of both tests.

- Increase or decrease in neutralizer concentration
- Options that represent a change in protocol and require preapproval from WHO:
  - Use of a different neutralizer
  - Dilution of the sample until the product is no longer at antimicrobial concentration, provided that the organism challenge level and method detection limit are still sufficient to demonstrate the necessary reduction.

During the test

The same three samples (A, B, C) as in the pre-test should be analyzed during the actual test for both spiked test waters for *E. coli* and the phages only, not *Cryptosporidium*.

Note: For each sample 3 consecutive dilutions are analyzed as two plates.

4.4.5 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.

4.4.5.1 Chemical addition products

For chemical addition (batch) systems, two (2) 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. Manufacturer 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 disinfectant 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.

Chemical additional products other than iodine (example: chlorine)

Sample Collection for GTW:

- 1 sample for test water characteristics
- 1 pre-treatment sample, neutralized and analyzed for organism of Table 3
- 6 post-treatment samples analyzed for organism of Table 3
- 2 post-treatment samples for product residual (one per triplicate run)

Sample Collection for CTW:

- 1 sample for test water characteristics
- 1 pre-treatment sample, neutralized and analyzed for organism of Table 3
- 6 post-treatment samples analyzed for organism of Table 3
- 2 post-treatment samples for product residual (one per triplicate run)

Iodine products

Sample Collection for GTW (20 ± 3ºC):

- 1 sample for test water characteristics according to Table 1
- 1 pre-treatment sample, neutralized and analyzed for organism of Table 3
- 3 post-treatment/effluent samples analyzed for organism of Table 3
- 1 post-treatment/effluent samples for product residual (one per triplicate run)

Sample Collection for GTW (35 ± 3ºC)

- 1 sample for test water characteristics according to Table 1
- 1 pre-treatment sample, neutralized and analyzed for organism of Table 3
- 3 post-treatment/effluent samples analyzed for organism of Table 3
- 2 post-treatment/effluent samples for product residual (one per triplicate run)

Sample Collection for CTW pH 9.0:

- 1 sample for test water characteristics according to Table 1
- 1 pre-treatment sample, neutralized and analyzed for organism of Table 3
- 3 post-treatment/effluent samples analyzed for organism of Table 3
- 1 post-treatment/effluent samples for product residual (one per triplicate run)

Sample Collection for CTW pH 5.0:

- 1 sample for test water characteristics according to Table 1
- 1 pre-treatment sample, neutralized and analyzed for organism of Table 3
- 3 post-treatment/effluent samples analyzed for organism of Table 3
- 1 post-treatment/effluent samples for product residual (one per triplicate run)

Dose based on 25th percentile of drop size

For products which are administered via dropper or similar delivery, 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.

4.4.5.2 Batch treatment systems (not flowing) 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:

- 1-2 pre-treatment samples (depending on GTW prep days) analyzed for organism of Table 3
- 1-2 samples (depending on GTW prep days) for test water characteristics
- 6 post-treatment samples analyzed for organism of Table 3

Sample Collection for CTW:

- 1-2 pre-treatment samples (depending on CTW prep days) analyzed for organism of Table 3
- 1-2 (depending on GTW prep days) sample for test water characteristics
- 6 post-treatment samples analyzed for organism of Table 3

4.4.5.3 Flowing systems

Sampling for microbiological organisms shall be conducted according to a sample schedule, 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 systems that experience reduced flow (clogging) with use, the sample schedule may include instruction on end of test if the system's flow or other indicator of 'end of life' has not been reached by Day 4. 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, for all or some identified volume of the Day 4 volume. This shall be referred to as a 'clogging point' sample.

Sample Collection for GTW:

- 2 pre-treatment sample analyzed for organism of Table 3 (1/day)
- 2 samples for test water characteristics (1/day)
- 6 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 pre-treatment sample analyzed for organism of Table 3
  - 3 post-treatment samples analyzed for organism of Table 3

Sample Collection for CTW:

- 2 pre-treatment sample analyzed for organism of Table 3 (1/day)
- 2 samples for test water characteristics (1/day)
- 6 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.

4.4.6 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-term 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.

4.4.7 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.

4.4.8 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 (measurable in terms of volume throughput, specific timeframe or other appropriate method).

4.4.9 Daily test capacity

For flowing systems, the operation manual may supply the daily capacity of the system and the system shall be run accordingly, but is targeted 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.

4.4.10 Leakage test

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

4.4.11 Seeding

To purge the system of the uncontaminated water, a sufficient flow of contaminated test water will be used (referred to as seeding). The systems shall be exposed to a minimum of 10 units void volumes or 1 L, 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.

4.4.12 Device cleaning

For systems, approaches to restore or maintain flow or performance identified in the Operator Manual shall be permitted during testing. The general test plan for the product type should provide direction on device cleaning during testing.

4.4.13 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 product specific test plan shall provide direction on component replacement during testing.

4.4.14 End of test

The technology-specific test plan 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 2-4 full days of flowing *potentially* followed by an accelerated clogging point initiated on Day 2 or 4 or clogging during the sample schedule prior to the final collection point.

4.4.15 Log reduction calculation

Testing shall be conducted simultaneously on the technology dictated number of replicates. At each microbiological sampling point, pre-treatment/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 pre-treatment/influent sample and replicate post-treatment/effluent as:

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

The geometric mean is defined as the nth 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 pre-treatment/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, 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}.

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

Example of calculating the log reduction:

Using an example pre-treatment/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 = log reduction

8.31 – 1.95 = 6.36 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.

4.4.16 Acceptable reduction deviation

All units of the product must continuously meet or exceed the reduction requirements shown in Table 3, except for the following acceptable allowance:

A maximum deviation of 0.2 $\log_{10}$ is acceptable for 25% of sample points at the two-star performance tier, and 0.4 $\log_{10}$ at the three-star performance tier. This means that for classification as a 2-star product, up to three of the twelve sample points can achieve a minimum reduction of 1.8 $\log_{10}$ for bacteria or protozoan cysts (instead of 2 $\log_{10}$), or 2.8 $\log_{10}$ for viruses (instead of 3 $\log_{10}$).

Each phage is treated separately for evaluating acceptable allowance, and the overall claim for viruses is based on the lower performing phage.

Additionally, the geometric mean of all microbiological reductions must meet or exceed the requirements of Table 3.

4.4.17 Records

All pertinent procedures and data shall be recorded and provided in a final report. The technology-specific test plan provides a list of the data that are to be reported.

4.4.18 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).

5 PROCEDURE

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

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.

6 INTERPRETATION OF RESULTS

The evaluation of household water treatment (HWT) technologies is based on the performance criteria set forth in *Evaluating household water treatment options: health-based targets and microbiological performance specifications* (WHO, 2011). These criteria were determined by applying the concept of tolerable burden of disease (acceptable risk) as set forth in the fourth edition of the WHO Guidelines for Drinking-water Quality (GDWQ, 2017). Using quantitative microbial risk models described in the GDWQ and assuming background levels of reference pathogens in untreated water, reductions of pathogens were calculated to meet health-based targets. From this, three categories of recommended performance (★★★, ★★ and ★) were developed, denoting descending order of performance.

Products classified as 3-star (★★★) are those that demonstrate at least 4 $\log_{10}$ reduction against bacteria and protozoa, and at least 5 $\log_{10}$ reduction against viruses. Products in the 2-star (★★) category are those that demonstrate at least 2 $\log_{10}$ reduction against bacteria and protozoa, and at least 3 $\log_{10}$ reduction against viruses. Products in the 1-star (★) category are those that meet the performance targets for at least 2-star (★★) for only two classes of pathogens.

Both 3-star and 2-star categories provide comprehensive protection against the three main classes of pathogens which cause diarrhoeal disease in humans. The use of these products is encouraged where there is no information on the specific pathogens in drinking-water (and a prudent approach is to protect against all three classes), or where piped supplies exist but are not safely managed.

In general, the use of products in the 1-star (★) category may be appropriate in targeted situations where the burden of diarrhoeal disease is high due to known classes of pathogens. For instance, although chlorination is ineffective against parasitic protozoa, it is known to be effective against bacteria and viruses. Thus, in a situation where the causative agent of disease is known, such as a cholera outbreak, chlorination can play an important role in improving the quality of water.

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^{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
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