

# **WHO International Scheme to Evaluate Household Water Treatment Technologies**

## **Testing Protocol for Ultraviolet Systems (with or without pre-filtration and/or disinfection addition) Version 4.0**

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## **Glossary**

Refer to Harmonized Testing Protocol: Technology Non-Specific Version 4.0.

## 1 UPDATES FROM PREVIOUS PROTOCOL

Refer to Harmonized Testing Protocol: Technology Non-Specific Version 4.0, Section 1, *Updates from Previous Protocol* for details on changes in the evaluation approach implemented from the previous round of evaluation.

## 2 PRODUCT INFORMATION REQUIREMENTS

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

### **Batch systems (static/no flowing treatment) 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 stated capacity (volume or time), if available

### **Flowing systems:**

- Flow rate
- Does the system include a UV transmittance sensor and alarm? If not, does it include any other product performance indicator, such as an indicator that the UV source is functioning?
- 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).

## 3 PURPOSE

Refer to Harmonized Testing Protocol: Technology Non-Specific Version 4.0.

## 4 METHOD

### **4.1 Replicate samples**

Three (3) units, where practical and available to representing different production lots, shall be selected and run as triplicates (3) in two (2) test waters.

The Scheme does not dictate that ultraviolet (UV) treatment devices must include a performance indicator (alarm). When present, an alarm provides a user an indication of whether the product is able to treat the source water, therefore the ability of the alarm to function properly and warn the user when the source water is not appropriate for UV disinfection by the device is critical to the performance. As such, for systems that do include an alarm, the approach will be such that the evaluation of performance shall be conducted in a test water with specifications that are just below the alarm set point (see Section 4.2).

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<sup>1</sup> Refer to the WHO website for the most recent EOI: [http://www.who.int/water\\_sanitation\\_health/water-quality/household/how-evaluation-scheme-works/en/](http://www.who.int/water_sanitation_health/water-quality/household/how-evaluation-scheme-works/en/)

## 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 General Test Water (GTW) representing high quality groundwater or rainwater, and a Challenge Test Water (CTW) with more aggressive water specifications to representing surface-water.

The GTW is not technology specific, and 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. For all test water analysis 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
- Total Organic Carbon (TOC): 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 TOC addition

#### **Tannic acid preparation**

Tannic acid addition shall be from a stock solution prepared as: 6 g of tannic acid powder dissolved in 1L of reverse osmosis (RO) or deionized water (DI) and 10 mg/L of sodium thiosulfate added to prevent oxidation and stabilize the solution. The resultant concentration of the sodium thiosulfate in the GTW is approximately 0.004 mg/L of sodium thiosulfate for every 1 mg/L TOC. 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 and 10 mg/L of sodium thiosulfate added to prevent oxidation and stabilize the solution. The resultant concentration of the sodium thiosulfate in the CTW is approximately 0.004 mg/L of sodium thiosulfate for every 1 mg/L TOC. 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

Reverse osmosis treated water shall be used as the base water and adjusted to meet the following characteristics presented in Table 1 for all UV technologies (with or without a performance indicator):

**Table 1. General Test Water Characteristics**

Constituent	Specification	Adjustment Materials (CAS# <sup>1</sup> )
Chlorine <sup>2</sup> (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)
Color U.V. Absorption 254 nm	Measure	None
Alkalinity <sup>3</sup> (mg/L as CaCO <sub>3</sub> )	100 ± 20 mg/L	Sodium bicarbonate (144-55-8)

<sup>1</sup> Chemical Abstract Service registration number. Refer to the definition section of this document for additional information

<sup>2</sup> 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

<sup>3</sup> Intended to buffer pH. Analyzed values may deviate from this range

#### 4.2.3 Challenge Test Water

Reverse osmosis treated water shall be used as the base water and adjusted to meet the following characteristics presented in Table 2.1 or 2.2. The CTW characteristics shall be determined by the presence of a UV sensor in the product to be tested.

For those systems without a UV sensor, CTW shall follow the characteristics of Table 2.1:

**Table 2.1. Challenge Test Water characteristics (systems without a UV sensor)**

Constituent	Specification	Adjustment Materials (CAS # <sup>1</sup> )
Chlorine <sup>2</sup> (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) <sup>3</sup>	15 ± 5 mg/L	Humic acid (6813-04-4, Supplier: Alfa Aesar)
Turbidity (NTU) <sup>3</sup>	30 ± 10 NTU	ISO spec. 12103-A2 fine test dust
Temp (°C)	20 + 1°C	Not applicable
TDS (mg/L)	1500 ± 150 mg/L	Sea Salts, Sigma Chemical Company (7732-18-5)
Alkalinity <sup>4</sup> (mg/L as CaCO <sub>3</sub> )	100 ± 20 mg/L	Sodium bicarbonate (144-55-8)
Color U.V. Absorption 254nm	Measure	None

<sup>1</sup> 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.

<sup>2</sup> Chemical Abstract Service registration number. Refer to the definitions of this document for additional information.

<sup>3</sup> 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.

<sup>4</sup> Intended to buffer pH. Analyzed values may deviate from this range.

For those systems with a performance indicator, CTW shall follow the characteristics according to Table 2.2.

**Table 3.2. Challenge Test Water characteristics (systems with a UV sensor)**

Constituent	Specification	Adjustment Materials (CAS # <sup>1</sup> )
Chlorine <sup>2</sup> (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 <sup>3</sup> (mg/L as CaCO <sub>3</sub> )	<0.05	None
Color U.V. Absorption 254nm	Until just below alarm activation <sup>4</sup>	Parahydroxybenzoic Acid (PHBA)

<sup>1</sup> 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.

<sup>2</sup> Chemical Abstract Service registration number. Refer to the definitions of this document for additional information.

<sup>3</sup> Intended to buffer pH. Analyzed values may deviate from this range.

<sup>4</sup> 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. This higher influent concentration is an option, not a requirement, for the manufacturer as the data may prove to be useful as other local protocols are known to require higher log reductions for bacteria.

**Table 4. Microbiological groups and reduction requirements**

Microbial group	Target pre-treatment challenge <sup>1</sup>	Minimum required reduction (log <sub>10</sub> )	
		★★★	★★
<b>Bacteria:</b> <i>Escherichia coli</i> (ATCC 11229)	≥10 <sup>6</sup> /mL	≥ 4	≥ 2
<b>Virus</b> <sup>2,3</sup> : MS-2 coliphage (ATCC 15597-B1), with host organisms: <i>E. coli</i> (ATCC 15597) or <i>Salmonella typhimurium</i> (WG49 NCTC 12484); and phiX-174 coliphage (ATCC 13706-B1) with host organisms: <i>E. coli</i> (ATCC 13706 or ATCC 700078)	≥10 <sup>5</sup> /mL	≥ 5	≥ 3
<b>Protozoa</b> <sup>4</sup> : <i>Cryptosporidium parvum</i> infectious oocysts	≥5x10 <sup>4</sup> /mL	≥ 4	≥ 2

<sup>1</sup> Pre-treatment 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. 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 for 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.

<sup>2</sup> Virus performance claim will be based on the lowest log reduction of the two phages.

<sup>3</sup> Host selection is dependent on method. Refer to Section 4.3.2 Organism Methods.

<sup>4</sup> For devices that rely solely on UV disinfection, protozoa will typically not be tested. If there is a decision to include the use of the protozoan group in the evaluation of a system that solely or in combination with another technology(ies), 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 as outlined in the Harmonized Protocol (WHO, 2018). UV disinfection technologies. Oocysts are much more sensitive to UV radiation than vegetative bacteria and will achieve a higher log reduction than what is achieved for *E. coli*. As such, for UV technologies, the protozoan performance will be based on the log reduction achieved by *E. coli*.



With respect to mechanical filtration/UV combination technologies, MS2 is not a good surrogate for oocysts for mechanical filtration (overly conservative), therefore if a UV product has mechanical filtration that is effective against oocysts, the total count method of evaluating oocyst reduction may be considered to evaluate the protozoan group.

#### 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 justification on the surrogate selection for enteric bacteria, enteric viruses and parasitic protozoa to be used for the evaluation of performance for these organism classes under the WHO Scheme, refer to the Harmonized Protocol V 2.0, Section 4.3.1 Selection of microorganisms.

#### 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 Part2: Enumeration of somatic coliphages)
    - *E. coli* host ATCC 700078 or ATCC 13706
    - Analyses shall be conducted in triplicate; the geometric mean and standard deviation for each water type and across all water types examined shall be reported.

- Samples shall be stored at a temperature between 1 – 8°C and processed within 24 hours of collection.
- Required sample volume to allow for processing in triplicate and a retain volume: 12 ml
- ***Cryptosporidium parvum*** oocysts (if elected to be used for the evaluation) shall be enumerated using
  - The test method in Annex A of NSF/ANSI 53: Drinking Water Treatment Units – Health Effects (2014); or
  - ISO 15553 Water quality - Isolation and identification of *Cryptosporidium* oöcysts and *Giardia* cysts in water.
  - These methods may be used when a system employs physical removal to reduce *Cryptosporidium parvum*.
    - Collected samples shall be stored at a temperature between 1 – 8°C and processed, stained and mounted within 24 hours.
    - Required sample volume to allow for processing in triplicate and a retain volume: 600 ml.
- ***Cryptosporidium parvum*** infectious oocysts (if elected to be used for the evaluation) 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

*Note: If the system includes a filter that is intended to remove Cryptosporidium to the required reduction levels, it may be possible to use an enumeration method (not infectivity/viability method). Refer to the Harmonized Protocol, Section 4.3.2 Organism methods for reference to appropriate method(s).*

## 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. The 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 Bacteriostatic or wetted contact material of concern

For products that employ a 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. (Refer to Section 4.4.4 Neutralization of the Harmonized Protocol V 2.0 for systems with bacteriostatic or disinfecting components).

### 4.4.4 Microbiological sample points

For batch treatment systems, sample collection shall be based on the product ‘treatment complete’ indicator, unless there is proper justification to approach differently. 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.

For plumbed systems, sample collection shall occur at start-up and at the end of each test day. Unless the design or product production would demand otherwise, a 3 L sample shall be collected. The 3 L will be subsampled for individual analyses.

See Section 5 for more testing details.

#### **Sample Collection for GTW**

- 1 pre-treatment sample analyzed for organism of Table 3
- 1 samples for test water characteristics
- 6 post-treatment/effluent samples analyzed for organism of Table 3

#### **Sample Collection for CTW**

- 1 pre-treatment sample analyzed for organism of Table 3
- 1 for test water characteristics
- 6 post-treatment/effluent samples analyzed for organism of Table 3

### **4.4.5 Conditioning**

For systems that require conditioning, conditioning shall be according to the operation manual. For UV batch technology, conditioning is expected to be short, such as a single batch. As such, conditioning shall use GTW for short-term conditioning (single day) with microbiological addition according to Table 3. This volume may meet seeding volume requirements or may require additional seeding per Section 4.4.9 Seeding. The volume used for conditioning shall not be counted as accumulated volume in determining test volume.

### **4.4.6 Cycling**

Cycling is the starting and stopping of flow as would occur in actual use. For non-flowing batch treatment systems, cycling would be expected to be the time for batch treatment, unless the intended use implies an alternate approach.

### **4.4.7 End of life**

For chlorine generator 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).

### **4.4.8 Daily test capacity**

The operator's manual may supply the daily capacity of the system and the system shall be run accordingly, but is targeted to not exceed 8 total hours of system operation in a single test day, unless there are special circumstances or the laboratory justifies a longer evaluation day.

UV technology batch systems would be limited in daily capacity based on treatment, with the expectation that the first batch of the day represents the more challenging treatment for the system as the UV bulb is not warmed up at start. As such, the where indicated in Table 5 as at the start of

the day, the sample collection shall be from the first batch treatment of the system, without seeding, unless product design necessitates the need for seeding or other daily start up procedures indicate otherwise.

#### **4.4.9 Seeding**

Although it is not expected that all UV products would require seeding, if product design necessitates seeding to purge the system of the uncontaminated water, a sufficient flow of contaminated test water will be used (referred to as seeding) to do so. In these cases, the systems shall be exposed to a single batch of microbiologically challenged water per Table 3 immediately prior to the full batch that is to be used for sample collection and evaluation.

#### **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 Device cleaning**

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

#### **4.4.12 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 test plan should provide direction on component replacement during testing.

#### **4.4.13 End of test**

The general test plan for the product type shall provide clear direction on ‘end of test’. The following may apply:

- Completion of the sampling schedule outline in Table 4.
  - The test is complete after the collection of samples (test capacity) according to Table 4.
  - The test capacity, as volume, shall be the accumulated volume during the test schedule.
- Clogging, (defined as a reduction of greater than 75% flow compared to initial flow) that cannot be restored with test permitted component replacement or cleaning procedures. The test capacity, as volume, shall be the accumulated volume during the time on test.
- As determined by signaling from product’s end of use indicator.

#### **4.4.14 Log reduction calculation**

Refer to Harmonized Testing Protocol: Technology Non-Specific Version 4.0.

#### **4.4.15 Acceptable reduction deviation**

Refer to Harmonized Testing Protocol: Technology Non-Specific Version 4.0.

#### **4.4.16 Records**

Refer to Harmonized Testing Protocol: Technology Non-Specific Version 4.0.

#### **4.4.17 Completeness**

Refer to Harmonized Testing Protocol: Technology Non-Specific Version 4.0.

## 5 PROCEDURE

1. Three systems (3) shall be tested simultaneously.
2. Systems shall be conditioned according to the product use instructions, when use instructions indicate conditioning prior to use.
3. Test waters shall be prepared daily and verified in accordance with Tables 1 and 2.
  - a. Testing in each test water (GTW and CTW) shall be run on separate days with all replicates of a single test water type 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.
4. Microbiologically spiked challenge water shall be prepared to meet the concentrations of Table 3 (all organism are compatible for combining).
5. Devices shall be operated according to the product's use instructions.
  - a. The laboratory technician(s) shall note batch processing times, any cleaning procedures, and other significant event throughout the testing.
  - b. If seeding is required: a complete batch of microbiologically spiked shall be treated PRIOR to the sample collection batch processing.
  - c. For batch systems, the first sample collection for each water type is to occur with the first cycle of the product for the day, unless the system design necessitates seeding prior to the sample collection batch. For plumbed systems, the first sample collection for each water type shall be the first water out of the product for the day, after any downstream plumbing is cleared of any water remaining from the previous day.
  - d. The second sample point for batch systems shall represent the final batch for the day based on daily test capacity (Section 4.4.8 Daily test capacity) unless time to treat does not allow for the first and second batch to be treated within an 8 hour test day (*Note: The laboratory may opt for a longer test day to allow for the sample collection with acceptance of this deviation to be the decision of WHO*). For plumbed systems, the second sample point shall be at the end of the test day, and shall be collected under steady-state flowing conditions.
  - e. The influent/pre-treatment microbiological sample shall be collected immediately from the microbiologically spiked test water after all effluent/post-treatment samples have been collected.
    - A duplicate volume shall be collected and retained as a backup for confirmation or retesting purposes, when necessary.
    - As the influent challenge water for all units was from a single source of microbiologically spiked prepped test water, a single influent sample shall be taken and for analyzed for the organisms of Table 3.
  - f. Microbiological influent/pre-treatment and effluent/post-treatment concentrations shall be presented in the final report.

**Table 5. Sampling schedule**

Test day: Collection point	Test water being treated	Microbiological tests	
		Influent	Effluent
Day 1: First batch or 3L of day/start up	GTW	Influent	Effluent
Day 1: Final batch or 3L of day/last water out	GTW	Influent	Effluent
Day 2: First batch or 3L of day/start up	CTW	Influent	Effluent
Day 2: Final batch or 3L of day/last water out	CTW	Influent	Effluent

## 6 INTERPRETATION OF RESULTS

Refer to Harmonized Testing Protocol: Technology Non-Specific Version 4.0.

### References

Refer to Harmonized Testing Protocol: Technology Non-Specific Version 4.0.