WHO International Scheme to Evaluate Household Water Treatment Technologies

Chlorine Chemical Disinfectant Technology
Version 4.1

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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.

Version 3.2 adds requirements for reducing the chlorine demand of the challenge organisms.

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)
- Usage instructions including required contact time (wait period prior to consumption)
- Chemical makeup of the product and the expected residual in the finished product
- Shelf life

3 PURPOSE

Refer to Harmonized Testing Protocol: Technology Non-Specific Version 3.0.

4 METHOD

4.1 Disinfectant residual pre-check

A disinfectant pre-check shall 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.

According to the manufacturer use instructions for waters with low turbidity, each device shall be operated twice (2) and dosed into reverse osmosis treated water at 20 + 3°C. The laboratory shall measure and report the temperature of the water.

There shall be no microbiological addition or microbiological performance evaluation. Unless otherwise specified in the use instructions , plastic vessels shall be used for the residual pre-check.

Samples will be collected and analysed for free chlorine according to NEN-EN-ISO 5667-3 and ISO 7393-1 and total chlorine according to SM 4500-Cl G or UNE-EN ISO 7393-1 at the manufacturer indicated treatment time[2]:. Sufficient volume to allow for a retain volume shall be collected. One sample volume shall be used for analysis and reporting. The backup volume shall be retained for confirmation or retesting purposes, when necessary.

4.2 Replicate samples for batch treatment systems

For chlorine chemical addition products, two (2) production lots shall be selected and run as triplicates (3) for each lot in two (2) test waters. If the product is manufactured as a continuous process and ‘lots’ are not appropriate, testing shall use a total of six (6) replicates of the continuous process product.

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

2 Note: contact times may vary for products with shorter or longer than typical disinfectant wait times prior to ‘treatment complete’

4.3 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 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 specifications. 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 analyses, 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): Not a specification for chemical products. Tannic acid addition to the test water volumes is to be weighed out based on carbon content 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.

4.3.1 TOC addition

Tannic acid preparation

Tannic acid addition shall be from a stock solution prepared as: 6 g of tannic acid powder dissolved in 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.

4.3.2 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:

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)
Chemical demand (as chlorine)	1.5 ± 0.1 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[3] (mg/L as CaCO3)	100 ± 20 mg/L	Sodium bicarbonate (144-55-8)

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

4.3.3 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:

Table 2. Challenge Test Water characteristics

Constituent	Specification	Adjustment Materials (CAS #[1])
Chlorine[2] (mg/L)	< 0.05	None
pH	9.0 + 0.2	Inorganic acid or base: Hydrochloric acid (7647-01-0) Sodium hydroxide (1310-73-2)
Chemical demand (as chlorine)	3.0 ± 0.2 mg/L	Tannic acid (1401-55-4, Supplier: Alfa Aesar)
Turbidity (NTU)	40 ± 10 NTU	ISO spec. 12103-A2 fine test dust
Temp (°C)	4 + 1°C	Not applicable
TDS (mg/L)	1500 ± 150 mg/L	Sea Salts, Sigma Chemical Company (7732-18-5)
Alkalinity[3] (mg/L as $CaCO_3$)	100 ± 20 mg/L	Sodium bicarbonate (144-55-8)

[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 Test Water may cause interference with analytical technique; measurements shall be made prior to addition of sea salts.

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

4.4 Microbiological organisms and challenge concentrations

Table 3 shows the organisms and American Type Culture Collection numbers (ATCC) used in evaluating performance for all technologies. Note that the pre-treatment challenge concentration for bacteria is higher than necessary to demonstrate Scheme performance targets.

Table 3. Microbiological 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

[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 minimum required reduction . Pre-treatment concentrations presented in table in harmonized units. Refer to Section 4.4.2 organism methods for actual volumes processed and method sensitivity.

[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 4.4.2 Organism Methods.

4.4.1 Selection of microorganisms

Refer to the Harmonized Protocol V 3.0, Section 4.3.1 Selection of microorganisms.

4.4.2 Organism production and assay 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 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. This may be achieved by growth in a nutrient broth at 37°C for 48 hours on a shaker. After the incubation period, wash the culture three times by centrifugation followed by suspension in phosphate buffered saline solution. Determine the concentration by plating on a suitable medium such as m-endo agar. The cell suspension may be kept up to 10 days if held at a temperature of approximately 4°C. If an alternative method is used,growth phases shall be determined using optical density (OD) and strain specific standard curves developed by the designated testing facility or plating on a suitable medium such as m-endo agar each time the cultures are prepared.

- Harvested cells shall be washed three times by repeating the following procedure three times:
  - Suspend cells in phosphate buffered saline.
  - Centrifugate to separate the cells and supernatant.
  - Discard supernatant.
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- Collected samples shall be stored at a temperature between 1 – 8°C and processed within 24 hours.
- Sample volume anticipated 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 Normative Annex 1 of NSF/ANSI 55: Ultraviolet Microbiological Water Treatment Systems (2020); *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, with the geometric mean for each water type and across all water types reported.
    - Samples shall be stored at a temperature between 1 – 8°C and processed within 24 hours of collection.
    - Required sample volume to allow for processing in triplicate and a retain volume: 12ml.
  - In addition to the above, the virus to be used for challenge testing shall undergo "washing" using either the Abcam PEG precipitation kit or Millipore Centricon Plus-70 centrifugal filter.

- **Coliphage phiX-174** (ATCC 13706-B1) shall be prepared and assayed using:
  - The method in Normative Annex 1 of NSF/ANSI 55: Ultraviolet Microbiological Water Treatment Systems (2020) for MS-2; with *E. coli* ATCC 700078 as the host; 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, with the geometric mean for each water type and across all water types reported.
    - Samples shall be stored at a temperature between 1 – 8°C and processed within 24 hours of collection.
    - Required sample volume to allow for processing in triplicate and a retain volume: 12ml.
  - In addition to the above, the virus to be used for challenge testing shall undergo "washing" using either the Abcam PEG precipitation kit or Millipore Centricon Plus-70 centrifugal filter.

4.5 Other test details

4.5.1 Untreated control

The microbiologically spiked test water to be used as the pre-treatment challenge concentration shall also serve as the untreated control. See Table 3 for concentrations.

4.5.2 Quality assurance / quality control (QA/QC)

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

4.5.3 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 samples, unless otherwise indicated. For chlorine disinfectants this shall include total and free available chlorine samples. 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.

4.5.4 Neutralization

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. Chlorine shall be neutralized using sodium thiosulfate.

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 five-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 (2x dilution) and process duplicate plates.
- The processing method, media, incubation conditions, etc. used should be according to Section 4.4.2 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.

4.5.5 Microbiological sample points

For chlorine technologies as 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. 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 plastic. Plastic is expected to have more disinfectant adsorption to the container walls and therefore would be considered to be most conservative. If possible, each test vessel shall be used only once and then discarded. If this is not practical, each test vessel shall be thoroughly disinfected and rinsed between each use.

Sample Collection for GTW

- 1 sample for test water characteristics
- 1 pre-treatment sample, neutralized and analyzed for organism per Table 3
- 6 post-treatment samples analyzed for organism of Table 3 (3 samples/lot)
- 6 post-treatment samples for product residual as Total and Free Available Chlorine

Sample Collection for CTW

- 1 sample for test water characteristics
- 1 pre-treatment sample, neutralized and analyzed for organism per Table 3
- 6 post-treatment samples analyzed for organism of Table 3 (3 samples/lot)
- 6 post-treatment samples for product residual as Total and Free Available Chlorine

4.5.6 Drop 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.

4.5.7 Daily test capacity

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

4.5.8 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.

4.5.9 End of test

For chemical addition products, end of test shall be completion of the test plan and collection of all data as indicated in Section 5 Procedure. The general test plan for the product type shall provide clear direction on 'end of test'. In the event that a chlorine disinfection chemical addition product included an indicator of water treatment 'complete', there shall be direction in the test plan for 'end of test' should the indicator not signal completion of treatment.

4.5.10 Log reduction calculation

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

4.5.11 Acceptable reduction deviation

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

4.5.12 Records

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

4.5.13 Completeness

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

5 PROCEDURE

1. Perform Section 4.1 Disinfectant residual pre-check.
2. Two (2) production lots shall be selected and run in triplicate (3) in two (2) test waters for each test organism.
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) may each be run as separate events, however, all replicates of a single test water type must be run simultaneously.
    b. Daily test water characteristics shall be sampled, analyzed and results provided in the final report. Sufficient volume to allow for a sample retain shall be collected. One shall be used for analysis and reporting. The backup volume shall be retained for confirmation or retesting purposes, when necessary.
4. Microbiologically spiked challenge water shall be prepared to meet the concentrations of Table 3 for the challenge organism(s).
5. The product shall be prepared and employed according the use instructions of the product instruction manual.
6. Test water that has been microbiologically spiked according to Table 3 shall be dosed with product according the use instructions of the product literature.
    a. Per the manufacturer's instruction for use, the product shall be added to the manufacturer indicated volume for two (2) lots, with each lot run in triplicate (3).
    b. After the addition of the product to the test water, the test contact/wait time shall begin (t=0).
    c. If agitation is indicated in the use instructions, the test vessel may be set on a rocker or shaking platform set to a setting that is consistent with the use instructions or may be agitated manually by the technician. Instructions may also dictate inversion which can be accomplished with test vessels that have stoppers.
    d. Laboratory technician shall record any observations of interest relative to the product dissolution, color, characteristics variation by lot, etc. in the laboratory bench sheets.

7. The microbiological post-treatment and disinfectant total and free available (active agent) samples identified in Section 4.5.5 shall be collected after completing the manufacturer dictated mixing and/or wait instructions. If the use instructions call for filtering the water through a cloth or other material, the entire test volume shall be filtered and a sample collected for analysis after filtration. Samples shall be neutralized immediately upon collection. Sufficient volume to allow for a retain volume shall be collected. One sample volume shall be used for analysis and reporting. The backup volume shall be retained for confirmation or retesting purposes, when necessary.
8. Pre-treatment samples shall be collected from the microbiologically spiked challenge water and analyzed to confirm pre-treatment concentrations (influent concentration) of challenge organisms. Pre-treatment samples shall also be collected for analysis of both the total and free available chlorine.
    a. As the pre-treatment challenge water for each set of triplicates was from a single source of microbiologically spiked prepped test water, a single pre-treatment sample per triplicate shall be sufficient.
    b. The pre-treatment microbiological shall be collected immediately from the microbiologically spiked test water after all post-treatment samples have been collected.
    c. Sufficient volume to allow for a retain volume shall be collected. One sample volume shall be used for analysis and reporting. The backup volume shall be retained for confirmation or retesting purposes, when necessary.
9. Microbiological pre-treatment and post-treatment concentrations shall be presented in the final report.

6 INTERPRETATION OF RESULTS

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

7 REFERENCES

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