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Glossary

1 UPDATES FROM PREVIOUS PROTOCOL
Refer to Harmonized Testing Protocol: Technology Non-Specific Version 3.0, 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)\(^1\) 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

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

4 METHOD
4.1 Disinfectant residual pre-check
If the product includes a disinfecting component, the disinfection shall be addressed under a separate protocol.

4.2 Replicate samples for coagulation and flocculation chemical addition products (tablets, sachets, drops)
For coagulation and flocculation 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.

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 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:

\(^1\) Refer to the WHO website for the most recent EoI: http://www.who.int/household_water/scheme/applicant/en/
- Chlorine (total): SM 4500-C1 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 weighed 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.

4.3.1 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.

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.

4.3.2 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

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Specification</th>
<th>Adjustment Materials (CAS#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine (mg/L)</td>
<td>&lt; 0.05</td>
<td>None</td>
</tr>
<tr>
<td>pH</td>
<td>7.0 ± 0.5</td>
<td>Inorganic acid or base:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydrochloric acid (7647-01-0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sodium hydroxide (1310-73-2)</td>
</tr>
<tr>
<td>Chemical demand (as chlorine)</td>
<td>1.5 mg/L</td>
<td>Humic Acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6813-04-4, Supplier: Alfa Aesar³</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>&lt; 1 NTU</td>
<td>No adjustment</td>
</tr>
<tr>
<td>Temp °C</td>
<td>20 ± 3°C</td>
<td>Not applicable</td>
</tr>
<tr>
<td>TDS (mg/L)</td>
<td>275 ± 225 mg/L</td>
<td>Sea Salts, Sigma Chemical Company (7732-18-5)</td>
</tr>
<tr>
<td>Alkalinity (mg/L as CaCO₃)</td>
<td>100 ± 20 mg/L</td>
<td>Sodium bicarbonate (144-55-8)</td>
</tr>
</tbody>
</table>

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 Target: 0.7 mL of 6 g/L humic acid stock per Section 3.4.1 TOC addition.
⁴ 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

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Specification</th>
<th>Adjustment Materials (CAS #)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine (mg/L)</td>
<td>&lt; 0.05</td>
<td>None</td>
</tr>
<tr>
<td>pH</td>
<td>9.0 ± 0.2</td>
<td>Inorganic acid or base:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydrochloric acid (7647-01-0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sodium hydroxide (1310-73-2)</td>
</tr>
<tr>
<td>Chemical demand (as chlorine)</td>
<td>3.0 mg/L</td>
<td>Tannic acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1401-55-4, Supplier: Alfa Aesar³)</td>
</tr>
<tr>
<td>Turbidity (NTU)³</td>
<td>30 ± 10 NTU</td>
<td>ISO spec. 12103-A2 fine test dust</td>
</tr>
<tr>
<td>Temp °C</td>
<td>4 ± 1°C</td>
<td>Not applicable</td>
</tr>
<tr>
<td>TDS (mg/L)</td>
<td>1500 ± 150 mg/L</td>
<td>Sea Salts, Sigma Chemical Company (7732-18-5)</td>
</tr>
<tr>
<td>Alkalinity (mg/L as CaCO₃)</td>
<td>100 ± 20 mg/L</td>
<td>Sodium bicarbonate (144-55-8)</td>
</tr>
</tbody>
</table>

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 Target: 0.5 mL to 0.6 mgL of 6 g/L tannic acid stock per Section 3.4.1 TOC addition

4 Intended to buffer pH. Analysed 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.

Table 3. Microbiological groups and reduction requirements

<table>
<thead>
<tr>
<th>Microbial group</th>
<th>Target pre-treatment challenge&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Minimum required reduction (log&lt;sub&gt;10&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protozoa: Cryptosporidium parvum infectious oocysts</td>
<td>≥ 5 x 10&lt;sup&gt;4&lt;/sup&gt; mL</td>
<td>≥ 4</td>
</tr>
</tbody>
</table>

<sup>1</sup> By enumeration method. Challenge oocysts should not be inactivated when evaluating physical removal processes since inactivation changes oocysts flexibility.

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 methods

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

_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
- 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.

4.5 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)


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. 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

Typically, there shall be no neutralization. However, if used, 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 (2x 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: \( \frac{C}{A} = X \)
- Neutralizer Toxicity: \( \frac{C}{B} = X \)

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.*

4.5.5 Microbiological sample points

For coagulation and flocculation 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, if 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.

Sample Collection for GTW

- 1 sample for test water characteristics
- 1 pre-treatment sample, analyzed for organism per Table 3
- 6 post-treatment samples analyzed for organism of Table 3 (3 samples/lot)
- 2 post-treatment samples for product residual (one per triplicate run) as Total and Free Available Chlorine or other disinfectant, if method is available.

Sample Collection for CTW

- 1 sample for test water characteristics
- 1 pre-treatment sample, for organism per Table 3
- 6 post-treatment samples analyzed for organism of Table 3 (3 samples/lot)
- 2 post-treatment samples for product residual (one per triplicate run) as Total and Free Available Chlorine or other disinfectant, if method is available.

4.5.6 Dose based on 25th percentile 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 general test plan for the specific technology provides 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 non-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

4.5.11 Acceptable reduction deviation

4.5.12 Records

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

5 PROCEDURE
1. Two (2) production lots shall be selected and run in triplicate (3) in two (2) test waters the test organism.
2. Test waters shall be prepared daily and verified in accordance with Tables 1 and 2.
   a. Testing in each test water (GTW and CTW) may each be run as separate events, however, all replicates of a single test water type must be run simultaneously.
   b. Daily test water characteristics shall be sampled, analyzed and results provided in the final report. Sufficient volume to allow for a sample retain shall be collected. One shall be used for analysis and reporting. The backup volume shall be retained for confirmation or retesting purposes, when necessary.
3. Microbiologically spiked challenge water shall be prepared to meet the concentrations of Table 3.
4. The product shall be prepped and employed according the use instructions of the product instruction manual.
5. Test water that has been microbiologically spiked according to Table 3 shall be dosed with product according the use instructions of the product literature.
   a. Per the manufacturer’s instruction for use, the product shall be added to the manufacturer indicated volume for 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.

6. The microbiological post-treatment and disinfectant total and free available (when active agent is a component of the technology) samples identified in Section 4.4.5 shall be collected after completing the manufacturer dictated mixing and/or wait instructions. When used, 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.

7. Pre-treatment challenge and disinfect total and free available (when active agent is a component of the technology) samples shall be collected from the microbiologically spiked challenge water and analyzed to confirm pre-treatment concentrations (influent concentration).

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 taken and for analyzed for the organisms of Table 3.

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


References