

Guide Standard
and
Protocol for Microbiological Evaluation
of
Drinking Water Treatment Devices



Water Quality India Association (WQIA), an independent, not-for-profit organization, is dedicated to provide guidelines for the evaluation of Drinking water treatment devices for conformity to safety standards for use in drinking water treatment.

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WQIA Protocol IP 100

Guide Standard and Protocol for Microbiological Evaluation of Drinking Water Treatment Devices

Protocol Developed by:

WQIA Task Force (Committee Comprising of Experts in household drinking water treatment system from the Industry)

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Guide Standard and Protocol for Microbiological Evaluation of Drinking Water Treatment Devices

1.0 Introduction

Point-of-use (POU) treatment of drinking water to alleviate health risks is gaining widespread application. Devices to reduce different forms of contaminants are being developed in accordance with water quality guidelines and health risks. Treatment devices that remove microbial contaminants are playing a significant role in reducing disease burden, especially in developing countries.

Given the variety of technologies available for point-of-use (POU) drinking water treatment, it is important to develop performance standards that guide the consumers to choose an appropriate treatment system.

The USEPA in 1987 proposed a comprehensive drinking water treatment guide standard for devices that perform microbiological treatment of water. This standard chose bacteria, viruses and disinfectant resistant parasites (cysts) as the key targets and the standard is relevant to water of unknown origin.

In developing & emerging countries like India, urban consumers in general have access to improved water sources through pipes. However the complexity of the water supply system which includes lack of 24 X 7 water supply, leaky pipes, illegal tapping, contaminated intermediate storage tank etc., lead to a situation of frequent microbial contamination of water supplies. In terms of physical-chemical composition, such urban water supplies are better when compared to waters of unknown origin. Except that at times high microbial load may be encountered in such waters.

In view of the above, the Water Quality India Association (WQIA) undertook the development of a separate microbial reduction standard that is relevant for the Indian residential water treatment market.

1.1 Disclaimer

WQIA shall not be responsible to anyone for the use of or reliance upon this Guide Standard by anyone. WQIA shall not incur any obligation or liability for damages, including consequential damages, arising out of or in connection with the use, interpretation of, or reliance upon this Standard.

This WQIA Standard provides basic criteria to promote product performance claims and protection of the public health. Provisions for mechanical and electrical safety have not been included in this Standard because governmental agencies or other national standards setting organizations already may provide such safety requirements.



2.0 Basic Principles

2.1 Definitions: A unit or system, in order to be called a Microbiological Water Treatment System (MWTS), must remove, kill, or inactivate all types of disease-causing microorganisms from the water, including bacteria, viruses, and protozoan cysts, so as to render the processed water safe for drinking. Therefore, to qualify, a MWTS must remove all types of challenge organisms to specified standards.

2.2 General Guide: The standard and protocol will be a general guide and, in some cases, may present only the minimum features and framework for testing. While basic features of the standard and protocol have been tested by others, it was not feasible to conduct full-fledged testing for all possible types of units. Consequently, protocol users should include pre-testing of their units in a testing rig, including the sampling techniques to be used. Where users of the protocol find good reason to alter or add to the guide in order to meet specific operational problems, to use an alternate organism or laboratory procedure, or to respond to innovative treatment units without decreasing the level of testing or altering the intent of the protocol, they should feel free to do so.

2.3 Performance-Based: The standard will be performance-based, utilizing realistic worst case challenges and test conditions, and shall result in water quality equivalent to that of a public water supply meeting the microbiological requirements.

2.4 Not to Cover Non-Microbiological Reduction Claims: The treatment of water to achieve specific chemical removal from water or other non-microbiological claims will not be a part of this standard.

2.5 Construction and Informational Exclusions: While the standard recommends safe, responsible construction of units with non-toxic materials for optimum operation, all such items and associated operational considerations are excluded as being beyond the scope of the standard. Included in the exclusion are materials of construction, electrical and safety aspects, design and construction details, operational instructions and information, and mechanical integrity testing. (There are many international standards available in USA/EU that can best be used for the processes/requirements of materials and structural integrity).

2.6 Continuity: This standard and protocol will be a living document, subject to revision and updating with the onset of new technology and knowledge. It is recommended that the responsible authorities for registration and drinking water quality review potential needs every two to three years and convene a task force upon need or upon request from the water quality industry, to review and update the standard and testing protocol.

2.7 Follow Standard Methods for Examination of Water and Wastewater, 22nd edition for the analysis of chemicals required in this Standard.



3.0 Treatment Units Covered

3.1 Universe of Possible Treatment Units: A review of treatment units that might be considered as microbiological purifiers discloses a number of different types covering treatment principles ranging from filtration and chemical disinfection to ultraviolet light radiation.

3.2 Coverage of This Standard: The initial coverage is limited, on a priority basis, to three basic types of microbiological water treatment systems or active components within their principal means of action as shown below. However several combinations can be visualized.

- 3.2.1 Mechanical Filtration - Pathogen exclusion/ removal technologies such as Ceramic Filtration Candles or Other Fine Filtration units or Membrane Units: filtration and adsorption and chemical anti-microbial activity if a chemical is included.
- 3.2.2 Halogenated Resins or Disinfectant feeding Units: Chemical disinfection with or without complementing pathogen exclusion technologies.
- 3.2.3 Ultraviolet (UV) Units: UV irradiation with possible add-on treatment for adsorption and filtration.

3.3 Application of Principles to Other Units: While only three types of units are described above in this standard, the principles and approaches outlined should provide a guide for the testing of any of a number of other types of units and/or systems for the microbiological purification of contaminated water.

3.4 Combination of water treatment technologies are allowed for evaluation under this protocol. If a combination technology is being evaluated the company shall specify the procedure they would like the laboratory to follow and make appropriate claims based only on the test results. (For example, there are systems marketed that incorporate two equally capable processes in series, such as Reverse Osmosis membranes and UV or Ultrafiltration. When such systems are to be tested by a laboratory, the manufacturer must decide as to which of the processes is to be tested and provide means to have the other processes disabled during the testing. The claims can however be made for the entire tested system, not the untested process in the system).



4.0 Performance Requirements

4.1 Microbiological Water Treatment System: In order to make the claim of “microbiological water treatment system,” units must be tested and demonstrated to meet the specifically required microbiological reduction requirements shown in Section 5.0 according to the test procedures described in Section 6.0 for the specific type of unit involved.

4.2 Chemical Health Requirements: Where silver or some other disinfectant chemical is used in a unit, that chemical shall be evaluated for its safety in drinking water using one of the following criteria.

- 4.2.1 Have been tested, evaluated and approved for use in drinking water treatment by an accredited independent global organization.
- 4.2.2 The concentration of the chemical and/or its by products in the effluent water must meet any Guide Level established by the World Health Organization or US EPA MCL or Guide Level established by BIS - IS 10500-2012 India standard to ensure not to constitute a risk to health from consumption or contact. The chemical shall be sampled and evaluated during the test procedures described in Section 6.0.

4.3 Stability of Disinfectant Chemical: Where a disinfectant chemical is used in the treatment unit, the stability of the chemical for disinfectant effectiveness should be sufficient for the potential shelf life and the projected use life of the unit based on manufacturer’s data. Where stability cannot be assured from historical data and information, the company shall provide stability assurance information. The company shall also specify in its operation manual the target period of use expiration date.

4.4 Performance Limitations:

Effective Lifetime

The manufacturer must provide an explicit indication or assurance of the unit’s effective use lifetime to warn the consumer of potential diminished treatment capability either through:

- a. Having the unit terminate discharge of treated water as tested per NSF/ANSI 53 - Performance Indication Device testing, or
- b. Sounding an alarm as tested per NSF/ANSI 53 - Performance Indication Device testing, or
- c. Providing single, explicit instructions for servicing or replacing units within the recommended use life (measurable in terms of volume throughput, specific time frame, or other appropriate method.)
- d. While it is not being required of UV units (especially POU systems) to have UV intensity monitors, a calendar timer and end of life indicator based on the length of ON time is required along with a lamp ON indicator light.



5.0 Microbiological Reduction Requirements

5.1 Pathogen exclusion technologies – Mechanical Filtration Units:

- a. At least 99.9999% Reduction (6 log) of *Klebsiella terrigena* (*Raoultella* MTCC2271) at an influent level of 50,000,000 cfu/100ml, and
- b. At least 99.9% Reduction (3 log) of MS2 Phage (ATCC 15597 B1) at an influent challenge level of 10,000 pfu/mL for all samples taken except the sample on Day 6.
 - o At the Day 6 sample point of the test with influent raised to 100,000 pfu/mL at least 99.99% reduction (4 log) shall be demonstrated.

Note - *Cryptosporidium parvum* oocyst testing is not required as Mechanical Filtration Units capable of removing MS2 phage will also remove cysts.

5.2 Halogen Resin/Disinfectant Feeding Units with a cyst Reduction Filter:

5.2.1 Option 1

- a. At least 99.9999% Reduction/inactivation (6 log) of *Klebsiella terrigena* at an influent level of 50,000,000 cfu/100ml.
- b. At least 99.9% Reduction/inactivation (3 log) of rotavirus Wa or SA11 at 1,000,000 pfu/1L for all samples taken except the 60% estimated capacity sample.
 - o At the 60% estimated capacity sample at least 99.99% reduction (4log) shall be demonstrated.

Note: For Iodine based resin units the higher log reduction sample shall be taken at the 70% estimated capacity sample instead of the 60% estimated capacity sample.

- c. At least 99% Reduction/inactivation (2 log) of *Cryptosporidium parvum* oocysts at an influent level of 50,000/L for all samples taken except the 60% estimated capacity sample.
 - o At the 60% estimated capacity sample of the test at least 99.9% reduction (3log) shall be demonstrated.

Note: For Iodine based resin units the higher log reduction sample shall be taken at the 70% estimated capacity sample instead of the 60% estimated capacity sample.

5.2.2 Option 2

- a. Alternatively MS2 Phage can be substituted in place of rotavirus Wa or SA11. When MS2 used at least 99.99% reduction (4log) of MS2 at an influent challenge level of 100,000 pfu/mL for all samples taken except the 60% estimated capacity sample.



- o At the 60% estimated capacity sample of the test with influent level raised to 1,000,000 pfu/mL at least 99.999% reduction (5 log) shall be demonstrated.

Note: For Iodine based resin units the higher log reduction sample shall be taken at the 70% estimated capacity sample instead of the 60% estimated capacity sample.

b. Alternatively Polystyrene Particle reduction can be substituted in place of live cysts if the system includes a filtration means for filtering out the cysts that may be present in the water. 99% reduction (2 log) of 3 micron polystyrene beads at a concentration of 50,000/liter shall be demonstrated for all samples taken except the 60% estimated capacity sample. (95% of these particles shall be in the range of 3+/-0.15microns).

- o At the 60% estimated capacity sample of the test at least 99.9% reduction (3 log) shall be demonstrated

Note 1: Polystyrene beads shall be 3 micron polystyrene micro spheres, Cat # 17147 FluoresbriteCarboxy YG 3 micron Microspheres: Poly sciences - USA,

Note 2: Option 1 and option 2 provide alternatives for challenging the test units. These options do not have to be taken in the entirety. For example Polystyrene particle reduction can be used to evaluate cyst reduction in option 1 instead of live cysts.

5.3 UV Units

- a. For UV systems that utilize a UV intensity monitor the NSF/ANSI 55 Class A testing protocol shall be used.
- b. For UV systems that do not use a UV intensity monitor - a UV intensity level of 50mJ/sqcm at 70% of lamp intensity following the NSF/ANSI 55 Class B testing protocol but using MS2 virus as the test organism shall be demonstrated.

Note 1: Follow the procedures indicated in Standard 55 for the test itself. Disregard the procedures outlined in Section 6 of this protocol for testing UV units.

Note 2: typical log reduction of MS2 at 50mJ/sqcm is 2 – 2.5 log.



6.0 Test procedures for mechanical filters and halogenated resins or disinfectant feeding units

6.1 Purpose

These tests are performed on ceramic filtration candles or fine filtration/membrane units, halogenated resin/disinfectant feeding units, in order to substantiate their microbiological removal/inactivation capabilities over the effective use life of the purifier as defined later in this section and, where a disinfectant chemical is used, to determine that said chemical is not present in the effluent at levels in excess of relevant safety/ regulatory limits.

6.2 Apparatus

Three production units of a type are to be tested simultaneously.

Design of the testing rig must parallel and simulate projected field use conditions. For plumbed-in units, a guide for design of the test rig may be taken from "Figure 1: Test Apparatus-Schematic" of ANSI/NSF Standard Number 53 "Drinking Water Treatment Units – Health Effects".

6.3 Test Water

The following challenge water shall be used to evaluate products under this section. Demineralized water shall be used as the starting point.

- a. shall be free of any chlorine or other disinfectant residual.
- b. pH range (7.0 – 8.0) except at the different sample points during the testing of iodine resin based devices as shown in Table 1b which require alternate pH values.

Note: the pH adjustment is only conducted during the 10 bed volume sampling event not the entire day. Total Organic Carbon (TOC): (1.5 – 2.5) mg/L (except it shall be adjusted to (4.5 – 5.5) mg/L during the 20% sampling time or on 2nd day of testing.

- c. Turbidity: (1.0 – 1.5) NTU except at the following sample time (100% or Day 10) the turbidity shall be adjusted to (14 – 16) NTU.

Note: the turbidity adjustment is only conducted during the 10 bed volume sampling event not the entire day.

- d. Temperature : (20°C to 30°C); except adjusted to (8 - 12°C & 38-42°C) during 40% & 80% sampling time (or 4th & 8th day) respectively and

Note: the temperature adjustment is only conducted during the 10 bed volume sampling event no the entire day.

- e. Total Dissolved Solids (TDS): 400 - 500 mg/L +/- 10%; max 300 mg/L total hardness as CaCO₃.



6.3.1 Recommended Materials for Adjusting Test Water Characteristics – All Chemicals used shall be Reagent Grade

- a) pH: inorganic acids or bases (i. e., HCl, NaOH, sodium bicarbonate);
- b) Total Organic Carbon (TOC): humic/tannic acids, sodium salt of humic acid;
- c) Turbidity: ISO Standardized test dust (fine is specification ISO 12103-A2);
- d) Total Dissolved Solids (TDS): sea salts, Sigma Chemical Co., S9883 (St. Louis, MO) or another equivalent source of TDS. Deionized or Reverse Osmosis treated water can be used to reduce the TDS.

6.4 Analytical Methods

6.4.1 Microbiological Methods

Methods used for microbiological analyses should be compatible with and equivalent to those shown in US EPA Microbiological Guide Standard, NSF/ANSI 55, NSF/ANSI 53, NSF P231, NSF P248 standards.

6.4.2 Chemical and Physical Methods

All physical and chemical analyses shall be conducted in accordance with procedures in Standard Methods for the Examination of Water and Wastewater, 22th Edition, American Public Health Association, or equivalent.

Note: newer additions or online additions are also acceptable.

6.5 Test Procedures

6.5.1 Procedure: Plumbed-In Units

- a. Install three production units of a type and condition each unit prior to the start of the test in accordance with the manufacturer's instructions using the test water without the addition of the microbial contaminants. The highest allowable test pressure shall be 60 psig (4.1 Bar) static. The flow rate shall not be artificially controlled by the testing laboratory. Measure the flow rate through of each unit.

Note 1: If the flow rate needs to be controlled for the system to function properly, the manufacturer shall include an automatic flow controlling means in each unit produced.

Note 2: Units designed to operate at much lower pressures can be tested at these lower pressures if such pressure restrictions are properly identified in product literature.



- b. Test waters shall have the defined characteristics continuously during the test duration except when indicated to be changed as shown in each of the Tables for different types of devices. These changes shall be maintained for at least 10 bed volumes and until the samples are taken as required. Afterwards the water quality shall be changed back to the general test characteristics.
- c. Start an operating cycle of 10 percent on, 90 percent off with a 15-to-40-minute cycle (Example: 3 minutes on, 27 minutes off) with the test water. This cycle shall be continued for not more than 16 hours per day (minimum daily rest period of 8 hours.) The total program shall extend to 120% of estimated volume capacity for halogenated resins or units and for 12 days for ceramic candles or mechanical filtration/membrane units. An alternate operating cycle of 20% on and 80% off can be used to accelerate the testing for volume capacity units but the test cannot be accelerated to complete in less than 12 days.

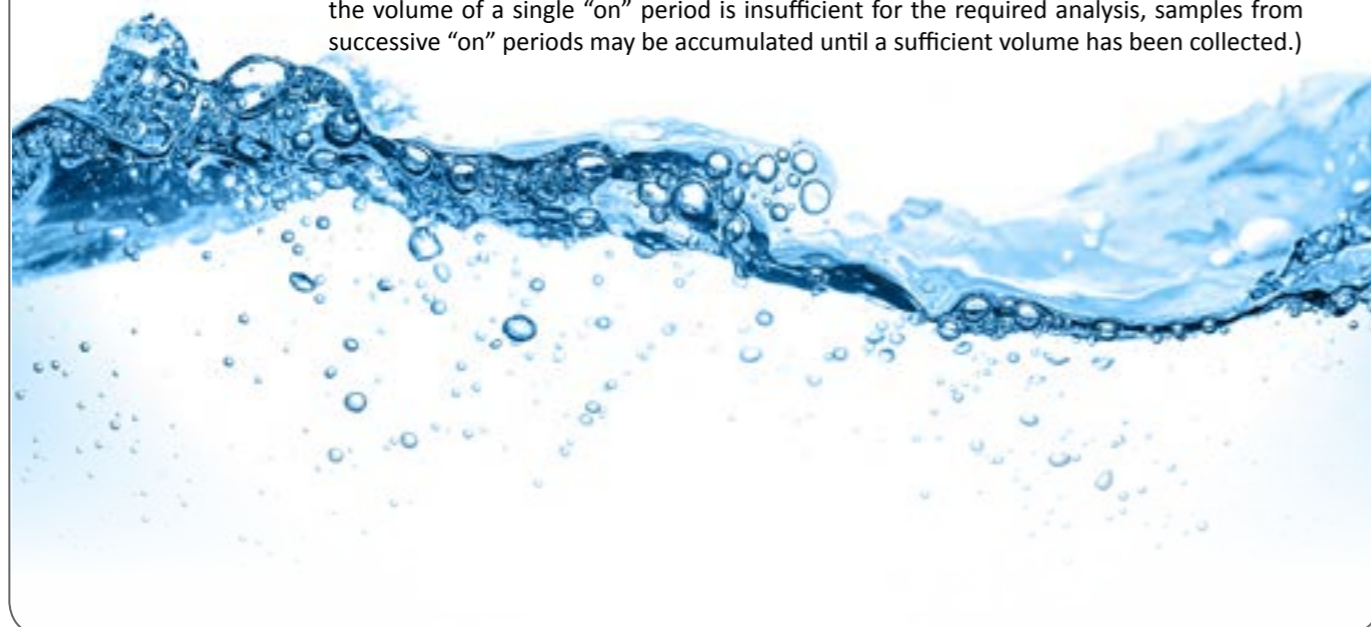
Note: (Membrane/other units with effluent storage tanks shall be operated to fill and dispense tanks continuously for 16 hours of operating time each day for 12 days).

- d. Use appropriate technique to prepare concentrated bacteria, virus, and protozoan suspensions as required. Feed these suspensions into the influent stream so as to achieve the influent concentrations specified in Section 2.5 in the following manner:
 - o At least “on” period(s) prior to the sampling “on” period.
 - o In the sampling “on” period when the sample actually will be taken.

Note 1: at least 10 unit void volumes of seeded water shall pass through the unit prior to sampling so as to provide adequate seasoning and uniformity before sample collection.

Note 2: For units with a storage tank – drain tank completely and begin feeding contaminated water until the tank fills. Repeat and sample water from the storage tank after it fills.

- e. Sampling - Samples of influent and effluent water at the specified sampling points shall be collected as shown below for the various units. All samples shall be collected in duplicate from the following water during the sampling “on” portion of the cycle and they shall be one “unit void volume” in quantity (or of appropriate quantity for analysis). Effluent samples shall be collected near the middle of the sampling “on” period (or the whole volume during one “on” period) except for samples following the specified “stagnation” period, for which sampling shall be conducted on the first water volume out of the unit. Each sample will be taken in duplicate and shall be retained and appropriately preserved, if required, for chemical or microbiological analysis in the event verification is required. (For units where the volume of a single “on” period is insufficient for the required analysis, samples from successive “on” periods may be accumulated until a sufficient volume has been collected.)



Note 1: When sampling a neutralizer may be required. 10% Sodium thioglycollate+ 14.6% Ringers Sodium Thiosulphate – Add 1 ml in 100 ml sample (to neutralise metal ions and chlorine)

Note 2: Samples are collected in duplicate but only one is required to be analyzed. The duplicate sample is retained if verification is needed.

Note 3: If excessive filter plugging occurs and it becomes impractical to collect duplicate samples only one effluent sample shall be collected.

Table 1(a). Sampling Plan: Halogenated Resins or Disinfectant Feeding Units (Non-Iodine Based)

Sample Point	Test Water				
	pH	TOC (mg/L)	Turbidity (NTU)	Temp. (°C)	TDS (mg/L)
Initial	7 – 8	1.5 – 2.5	1.5 – 2.5	20 – 30	400 – 500
20%	7 – 8	4.5 – 5.5	1.5 – 2.5	20 – 30	400 – 500
40%	7 – 8	1.5 – 2.5	1.5 – 2.5	8 – 12	400 – 500
After 48 hour stagnation	7 – 8	1.5 – 2.5	1.5 – 2.5	20 – 30	400 – 500
60%	7 – 8	1.5 – 2.5	1.5 – 2.5	20 – 30	400 – 500
80%	7 – 8	1.5 – 2.5	1.5 – 2.5	38 – 42	400 – 500
After 48 hour stagnation	7 – 8	1.5 – 2.5	1.5 – 2.5	20 – 30	400 – 500
100%	7 – 8	1.5 – 2.5	13.5 - 16.5	20 – 30	400 – 500
120%	7 – 8	1.5 – 2.5	1.5 – 2.5	20 – 30	400 – 500

Note 1: Analysis of influent test water characteristics, active agents (if necessary), and microbes shall be analyzed at each sample point.

Note 2: Required to test at 120% life point unless the unit shuts the flow of water from the device.



Table 1(b). Sampling Plan: Iodinated Resins or Units

Sample Point	Test Water				
	pH	TOC (mg/L)	Turbidity (NTU)	Temp. (°C)	TDS (mg/L)
Initial	7 – 8	1.5 – 2.5	1.5 – 2.5	20 - 30	400 – 500
20%	7 – 8	4.5 – 5.5	1.5 – 2.5	20 - 30	400 – 500
40%	7 – 8	1.5 – 2.5	1.5 – 2.5	8 - 12	400 – 500
After 48 hour stagnation	7 – 8	1.5 – 2.5	1.5 – 2.5	20 - 30	400 – 500
60%	7 – 8	1.5 – 2.5	1.5 – 2.5	20 - 30	400 – 500
70%	9 ± 0.2	1.5 – 2.5	1.5 – 2.5	20 - 30	400 – 500
80%	7 – 8	1.5 – 2.5	1.5 – 2.5	38 - 42	400 - 500
After 48 hour stagnation	7 – 8	1.5 – 2.5	1.5 – 2.5	20 - 30	400 - 500
90%	5 ± 0.2	1.5 – 2.5	1.5 – 2.5	20 - 30	400 - 500
100%	7 – 8	1.5 – 2.5	13.5 - 16.5	20 - 30	400 - 500
120%	7 – 8	1.5 – 2.5	1.5 – 2.5	20 - 30	400 - 500

Note 1: Analysis of influent test water characteristics, active agents (if necessary), and microbes shall be analyzed at each sample point.

Note 2: required to test at 120% life point unless the unit shuts the flow of water from the device.



Table 1 (c) Sampling Plan: Pathogen exclusion technologies - Ceramic Candles or Mechanical Filtration/Membrane Units

Sample Point	Test Water				
	pH	TOC (mg/L)	Turbidity (NTU)	Temp. (°C)	TDS (mg/L)
Initial	7 – 8	1.5 – 2.5	1.5 – 2.5	20 - 30	400 - 500
Day 2	7 – 8	4.5 – 5.5	1.5 – 2.5	20 - 30	400 - 500
Day 4	7 – 8	1.5 – 2.5	1.5 – 2.5	8 – 12	400 - 500
After 48 hour stagnation	7 – 8	1.5 – 2.5	1.5 – 2.5	20 - 30	400 - 500
Day 6 (middle)	7 – 8	1.5 – 2.5	1.5 – 2.5	20 - 30	400 - 500
Day 8 (near end)	7 – 8	1.5 – 2.5	1.5 – 2.5	38 - 42	400 - 500
After 48 hour stagnation	7 – 8	1.5 – 2.5	1.5 – 2.5	20 - 30	400 - 500
Day 10	7 – 8	1.5 – 2.5	13.5 - 16.5	20 - 30	400 - 500
Day 12	7 – 8	1.5 – 2.5	1.5 – 2.5	20 - 30	400 - 500

Note: All days are “running days” and exclude stagnation periods. When the units contain silver or similar chemicals, residual and by-products will be measured at each microbiological sampling point.

Note 1: Analysis of influent test water characteristics, active agents (if necessary), and microbes shall be analyzed at each sample point.

6.5.1.1 Alternate Sampling Plans:

Since some laboratories may find it inconvenient to test some units on a 16-hour “on” / 8-hour “off” cycle, two alternates are recognized:

- a. Go to a shorter operational day but lengthen the days of the test proportionally.
- b. Use up to 20 percent “on” / 80 percent “off” for a proportionally shorter operational day.
- c. Sampling points must be appropriately adjusted in any sampling plan.



6.5.1.2 Analyses and Monitoring:

- a. Microbiological sampling and analysis shall be conducted of the specified influent and effluent sampling points during each indicated sampling period.
- b. Test Water Monitoring: The specified parameters of the various test waters (see section 6.3) will be measured and recorded at each microbiological sampling point; the specified parameters will be measured at least once in non-sampling days when the units are being operated.
- c. When a unit contains a halogen or silver, the active agent residual will be measured in the effluent at each microbiological test (sampling) point.

6.5.1.3 Special Provisions for Ceramic Candles or Units:

- a. Provisions for slow flow: Ceramic units may be subject to clogging and greatly reduced flow over the test period. An attempt should be made to maintain manufacturer rated or claimed flow rates, but even at reduced flows, the sampling program set forth in section 6.5.1 shall be maintained.
- b. Cleaning of ceramic units: Units should be cleaned according to manufacturer's directions. A minimum of two cleanings should occur during the period of test (in order to prove the unit's durability through the cleaning procedure.) However, near the time of microbiological sampling, the units should not be cleaned until after the sampling. Further, no anti-microbial chemical (for cleaning or sanitizing) may be applied to the units during the test period unless the manufacturer specifies the same as part of routine maintenance.

6.5.1.4 Halogenated units or Disinfectant Feeding units with mechanical filtration processes separate from the microbiological disinfection components shall have the mechanical filtration components replaced or serviced when significant flow reduction (clogging) occurs in accordance with the manufacturer's instructions in order to maintain the test flow rate. Units with non-removable mechanical filtration components will be run until flow is below that considered acceptable for consumer convenience. (If premature clogging presents a problem, some specialized units may require a customized test plan).



6.5.2 Procedure: Non-Plumbed Units (Gravity Type)

- 6.5.2.1 General: The basic procedures given in section 6.5.1 shall be used with necessary adaptations to allow for the specific design of the unit. In any event, the testing procedures shall provide a test challenge equivalent to those for plumbed-in units.
- 6.5.2.2 Test conditions and apparatus should be adapted to reflect proposed or actual use conditions in consultation with the manufacturer, including flow rate and number of people served per day. In some cases, variable flow or other non-standard conditions may be necessary to reflect a worst-case test.
- 6.5.2.3 Filter plugging can make it difficult for the product to reach its estimated capacity. When plugging occurs the length of the testing may need to be increased to reach capacity. If the product plugs prior to reaching capacity re-testing at a lower capacity may be required. Pre-filters may be changed and cleaned to assist with plugging issues if they are part of the system.
- 6.5.2.4 At the challenge points fill the top reservoir with the challenge water and microorganisms one time to condition the unit. The second fill with microorganisms shall be used for analysis.

Note 1: Influent water modifications such as higher, TOC, Turbidity, Temperature, pH, shall only be conducted during the sampling event.

Note 2: Correct challenge water temperature shall be added to the upper reservoir during sampling events. Do not try to maintain the temperature in the reservoir.

7.0 Acceptance and Records

- 7.1 To qualify as a microbiological water treatment system, three production units of a type must continuously meet or exceed the reduction requirements of Section 4.0.
- 7.2 Where silver or some other chemical is used in the unit, concentrations in the effluent water must not constitute a threat to health as per IS10500-1991 or US EPA MCL or WHO limits.

Note: Scaling up or down - Where a manufacturer has several similar units using the same basic technology and parallel construction and operation, it may sometimes be appropriate to allow the test of one unit to be considered representative of others. Where any serious doubt exists, all units of various sizes may require testing. A "rule of three" is suggested as a matter of judgment. Scaling up to three times larger or one-third, based on the size of either the test unit or of its operative element, may be allowed. However, for UV units, any size scale-up must be accompanied by a parallel increase in radiation dose.

Annexure 1- Example Bacteria preparation and assay- users are not required to follow this procedure it is only provided as an example.



Materials:**A.1 Equipment and Accessories-**

- Vortex mixer
- Millipore vacuum pump
- 0.45 µ sterile Millipore membrane filters (Millipore HAWP 04700)
- Sterile syringes and forceps
- Pipettes
- Incubator
- Laminar air flow
- pH meter

A.2 Chemicals and Reagents-**A.2.1 Test organism Klebsiellaterrigena suspension-**

The level of bacteria per spike should be approximately $\sim 5 \times 10^7/100\text{ml}$, as a 7 log₁₀ spike is required for determining up to 6 log₁₀ reductions.

A.2.2 Neutralizer-

10% Sodium thioglycollate+ 14.6% Ringers Sodium Thiosulphate – Add 1 ml in 100 ml sample (to neutralise metal ions and chlorine). Alternately D/E medium could be used as a neutralizer.

A.2.3 Sterile MacConkey's Agar Medium**A.2.4 Sterile Tryptic Soy Agar Medium****A.2.5 Sterile Tryptic Soy Broth Medium****A.2.6 Sterile Saline- 0.85% NaCl****A.3 Procedure:****A.3.1 Bacterial Culture Maintenance & Preparation For Testing Purposes-**

The Growth State of the test bacteria is critical as this affects sensitivity and resistance to disinfectants. **Consequently, only bacteria in the stationary phase should be used.** The test organism should be washed and suspended in sterile saline before addition to the challenge water.



1. For this purpose, the test organism or is to be maintained on Trypticase Soy Agar (TSA) slants and checked using MacConkey's agar for purity.
2. The culture is to be held frozen in glycerol (or equivalent method) containing media at -800°C for a period of not more than 6 months and is to be revived & preserved again.
3. The culture is to be passed once in TSB for 15-18 hours and incubated at 37°C .
4. The resultant suspension is to be recovered by centrifugation at $3000 \times g$ for 10 minutes. Or an isolated colony is to be suspended in sterile saline and adjusted to get the desired OD. Alternately, culture could be recovered from overnight plate directly in saline (taking care not to scrape out agar) and the OD adjusted to 0.8-1.0.
5. The supernatant is to be discarded and the cells washed with sterile saline and resuspended in sterile saline.
6. Adjust the culture density of this suspension, using spectrophotometer (appropriately blanked), to attain an OD_{600nm} of 0.8 to 1.0 ($\sim 10^9\text{cfu/ml}$). Add sufficient volume of this to the spike water to attain the desired seed value.

A.3.2 Preparation of Spike Water- (two device cycle volumes)

1. The spike water is prepared using the test water as mentioned above (section 3).
2. Seed appropriate volume of culture suspension of the test organism to get a final concentration of $\sim 5 \times 10^7/100 \text{ ml}$.
3. The spike water is to be mixed well before use.
4. Independently establish that the spike water has no adverse impact on the survival or proliferation of the bacteria.
5. Prepare fresh spike water daily and ensure day storage of spike water does not affect viability or reduce the spike numbers.

A.3.3 Conditioning of Purification Device, Spiking and Sample Collection-

1. Make sure that the device to be tested has seen at least two device cycles of test water to reduce Particles / Dirt etc. entering the output samples. This is known as the conditioning cycle. Adjust the flow rate of the test device during this conditioning cycle if desired.
2. Check that the Purification Device has been fixed properly and there is no leakage from the sides and output water comes out only from the outlet terminal. Remove and drain as much water as possible from the device taking care not to accidentally contaminate them.
3. Now add the spiked water in to the inlet/ input port.
4. Sample collection has two stages:



- a) Collect the sample from the seeded water before it sees the device. This is the Influent sample, which will estimate the initial load of organisms to start with.
 - b) Collect the test sample, after ensuring that the sufficient volume of seeded water has passed through. This is referred to as effluent sample. Collect two 500 ml effluent samples. Analyse one sample retain the other.
5. This can be ensured by collecting the sample after the seeded water has started passing through and completed 10 bed volumes. Ensure that at least two samples of about 110 ml each are collected from a single spike for analysis. Add suitable neutraliser (Section 3) immediately on collection.
 6. After the spike water equivalent to at least 10 bed volumes of the device has passed through and all effluent samples collected, start passing through the test water without the test organism
 7. This procedure (Step 4-6) is to be carried out all intended test organisms along with the bacteria.
 8. Take care that there is no residual disinfectant in any of the samples.
 9. The devices may have a dead volume, so please ensure that sampling is done when at least 10 bed volumes of spike water has passed through the unit and starts coming out in the effluent.

A.3.4 Analysis

Input sample and output samples must go through all the steps involved in analysis in identical manner. Sample number and type should be written on labels during all steps of processing.

Bacteriological testing must take place within 1 hour of collecting the sample and it is done in 2 ways for estimation of actual log reduction.

A. Standard Membrane Filtration Method.

1. To establish the log reduction, 100 ml of the effluent should be tested.
2. 100 ml of the sample is to be passed through a sterile 0.45-micron filter, which is then placed on a pre-poured MacConkey's Agar plate and incubated at 37°C for 24-48 hrs. (mFC, MEndo agars can optionally be used as long as recognised by the regulatory bodies)

B. Standard Pour Plate Method.

1. Prepare a serial dilution of the test sample in saline.
2. Plate out undiluted and -2 on MacConkey's as per standard pour plate method (1 ml per dilution per plate) and incubate at 37°C for 24hrs. This should be done in duplicate.
3. Appropriate positive control (plating neat *E. coli* suspension) and negative controls (membrane, medium & saline controls) must be kept during each challenge experiment to eliminate errors due bacterial strain, media or diluent.
4. All standard precautions to be followed for bacterial testing must be strictly adhered to.

A.4 Calculations:

The bacterial log reduction is given as follows-

Input Load = ____ cfu/100ml

Log10 = ____

Output Load (mean) = ____ cfu/100ml

Log10 = ____

Log10 Reduction = Log10 (Input) – Log10 (Output)

Annexure 2 – MS2 phage preparation and assay

Use the appendix in NSF/ANSI Standard 55 for such preparation and assay.

Rotavirus

The rotaviruses are best grown in the MA-104 cell line. Since both viruses can be assayed on the MA-104 cell line a challenge test may consist of equal amounts of both viruses as a mixture (i.e., the mixture must contain at least 1.0×10^7 /ml of each virus). Assays may be as plaque forming units (PFU) or as immunofluorescence foci (IF) (Smith and Gerba, 1982, In Methods in Environmental Virology, pp. 15-47). Each dilution will be assayed in triplicate.

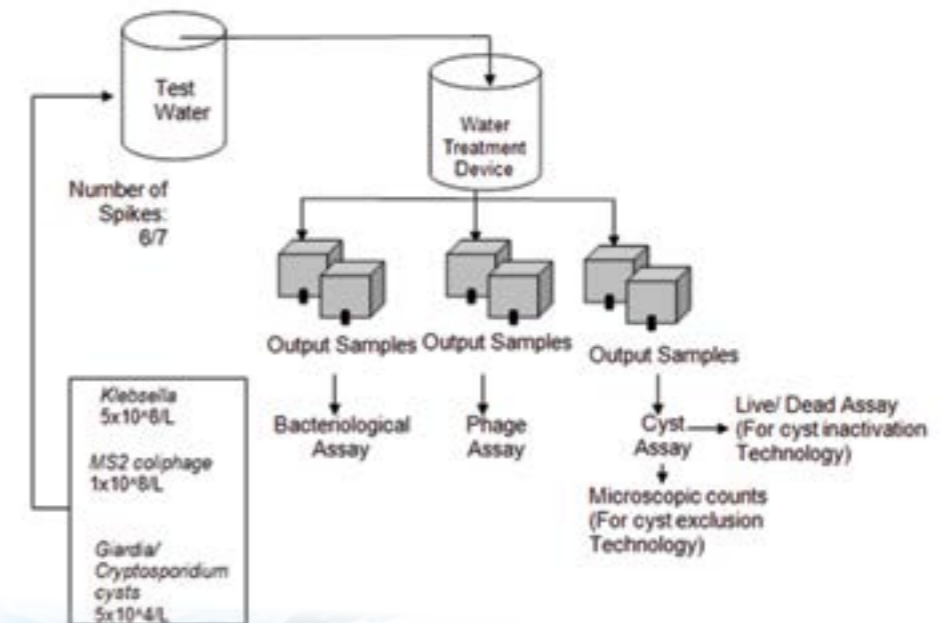
Annexure 3 - Methodology for *Cryptosporidium* Cysts

Use the Appendix in NSF/ANSI Standard 53 for the preparation and analysis of *Cryptosporidium* Cysts

Annexure 4 – METHODS FOR EVALUATING FILTRATION UNITS PERFORMING *CRYPTOSPORIDIUM* INACTIVATION

Use the appendix in NSF/ANSI Standard 53 for preparation and analysis of the latex particles.

Annexure 5 - Scheme for Testing



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