

**UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY**

**Registration Division
Office of Pesticide Programs
Criteria and Standards Division
Office of Drinking Water**

**GUIDE STANDARD AND PROTOCOL FOR
TESTING MICROBIOLOGICAL WATER PURIFIERS**

**Report of Task Force
Submitted April, 1986
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PREFACE

The task force began deliberations in July, 1984 and submitted its initial report in April, 1986. The task force included a broad multi-disciplinary group of experts representing the interest areas of academic and governmental research, product evaluation, development and testing, manufacturer's product registration, and governmental enforcement.

The report was provided for public comments in May, 1986. A review subcommittee was constituted to prepare a response to the public comments and to revise the report, as herewith submitted. Additional revision has been provided in response to review by the Scientific Advisory Panel (Federal Insecticide, Fungicide, and Rodenticide Act).

The recommended guide standard and testing protocol was developed to be useful in a number of ways, not only for governmental but also for industrial and consumer purposes:

- as a basic framework, starting point for the testing and evaluation of microbiological water purifiers for EPA registration;
- as a guide to the acceptance of water treatment units for compliance with Safe Drinking Water Act requirements where point of use units may be needed temporarily to treat a contaminated public water supply or for emergency situations, but not for use in extreme overseas situations or for the conversion of waste water to microbiologically potable water;
- as a testing guide to manufacturers wishing to have their units considered as microbiological water purifiers, whether registered or not, and for the evaluation of such testing data;
- as a guide to consumers regarding what they can expect from microbiological water purifiers tested according to this standard and protocol;
- to assist in the research and development of microbiological treatment units for possible military applications.

I want to thank the expert members of the task force for their participation in this work and particularly the chairmen of three work groups:

Charles Gerba: Microbiological Challenges
Richard Tobin: Physical, Chemical and Operational Challenges
Frank A. Bell, Jr.: Testing Protocol

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SECTION 1: GENERAL

1.1 Introduction

The subject of microbiological purification for waters of unknown microbiological quality repeatedly presents itself to a variety of governmental and non-governmental agencies, consumer groups, manufacturers and others. Examples of possible application of such purification capabilities include:

- backpackers and campers
- non-standard military requirements
- floods and other natural disasters
- foreign travel and stations (however, not for extreme contamination situations outside of the U.S.)
- contaminated individual sources, wells and springs (however, not for the conversion of waste water to microbiologically potable water)
- motorhomes and trailers

Batch methods of water purification based on chlorine and iodine disinfection or boiling are well known, but many situations and personal choice call for the consideration of water treatment equipment. Federal agencies specifically involved in responding to questions and problems relating to microbiological purifier equipment include:

Registration Division, Office of Pesticide Programs (OPP), Environmental Protection Agency (EPA): registration of microbiological purifiers (using chemicals);

Compliance Monitoring Staff, EPA: control of microbiological purifier device claims (non-registerable products such as ultra-violet units, ozonators, chlorine generators, others);

U.S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL), U.S. Army Natick Research and Development Center and other Army and military agencies: research and development for possible field applications;

Criteria and Standards Division, Office of Drinking Water (ODW), EPA: Consideration of point-of-use technology as acceptable technology under the Primary Drinking Water Regulations; consumer information and service;

Drinking Water Research, Water Engineering Research Laboratory (WERL), EPA; responsible for water treatment technology research;

Microbiology Branch, Health Effects Research Laboratory (HERL), EPA; responsible for study of health effects related to drinking water filters.

A number of representatives of the above mentioned agencies provided excellent participation in the task force to develop microbiological testing protocols for water purifiers. Major participation was also provided by the following:

- a technical representative from the Water Quality Association;
- a technical representative from the Environmental Health Center, Department of Health and Welfare of Canada; and
- an associate professor (microbiology) from the University of Arizona.

1.2 Basic Principles

1.2.1 Definition: As set forth in EPA Enforcement Strategy and as supported by a Federal Trade Commission (FTC) decision (FTC v. Sibco Products Co., Inc., et al., Nov. 22, 1965), a unit, in order to be called a microbiological water purifier, 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 microbiological water purifier must treat or remove all types of challenge organisms to meet specified standards.

1.2.2 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, 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. For example, the OPP Registration Division might find it necessary to amend the guide somewhat for different types of treatment units. Another example would be ultraviolet (U.V.) units, which may have specific requirements in addition to the guide protocol.

1.2.3 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 and intent of the National Primary Drinking Water Regulations.

- 1.2.4 Exceptions: A microbiological water purifier must remove, kill or inactivate all types of pathogenic organisms if claims are made for any organism. However, an exception for limited claims may be allowed for units removing specific organisms to serve a definable environmental need (i.e., cyst reduction units which can be used on otherwise disinfected and microbiologically safe drinking water, such as a disinfected but unfiltered surface water containing cysts. Such units are not to be called microbiological water purifiers and should not be used as sole treatment for an untreated raw water.)
- 1.2.5 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. National Sanitation Foundation (NSF) Standards 42 (Aesthetic Effects) and 53 (Health Effects) provide partial guides for chemical removal and other claims testing.
- 1.2.6 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 performance testing.
- 1.2.7 Research Needs Excluded: The guide standard and protocol must represent a practical testing program and not include research recommendations. For example, consideration of mutant organisms or differentiation between injured and dead organisms would be research items at this time and not appropriate for inclusion in the standard.
- 1.2.8 Not To Consider Sabotage: Esoteric problems which could be presented by a variety of hypothetical terrorist (or wartime) situations, would provide an unnecessary complication, and are not appropriate for inclusion in the standard.
- 1.2.9 Continuity: The guide 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 reconvene the task force upon need or upon request from the water quality industry, to review and update the standard and testing protocol.
- 1.3 Treatment Units Coverage
- 1.3.1 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.

- 1.3.2 Coverage of This Standard: In view of the limited technical data available and in order to expedite the work of the task force, the initial coverage is limited, on a priority basis, to three basic types of microbiological water purifiers or active components with their principal means of action as follows:
- 1.3.2.1 Ceramic Filtration Candles or Units (may or may not contain a chemical bacteriostatic agent): filtration, and adsorption, and chemical anti-microbial activity if a chemical is included.
 - 1.3.2.2 Halogenated Resins and Units: chemical disinfection and possibly filtration. [Note: While not included in this guide standard, halogen products for disinfection or systems using halogen addition and fine filtration may be tested using many of its elements, i.e., test water parameters, microbiological challenge and reduction requirements, analytical techniques and other pertinent elements.]
 - 1.3.2.3 Ultraviolet (UV) Units: UV irradiation with possible add-on treatment for adsorption and filtration (not applicable to UV units for treating potable water from public water supply systems).
- 1.3.3 Application of Principles to Other Units: While only three types of units are covered in this standard, the principles and approaches outlined should provide an initial guide for the testing of any of a number of other types of units and/or systems for the microbiological purification of contaminated water.

SECTION 2: PERFORMANCE REQUIREMENTS

2.1 Microbiological Water Purifier

In order to make the claim of "microbiological water purifier," units must be tested and demonstrated to meet the microbiological reduction requirements of Table 1 according to the test procedures described in Section 3 for the specific type of unit involved.

2.2 Chemical Health Limits

Where silver or some other pesticidal chemical is used in a unit, that chemical concentration in the effluent water must meet any National Primary Drinking Water Maximum Contaminant Level (MCL), additional Federal guidelines or otherwise be demonstrated not to constitute a threat to health from consumption or contact where no MCL exists.

2.3 Stability of Pesticidal Chemical

Where a pesticidal 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, additional tests will be required.

2.4 Performance Limitations

2.4.1 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, or
- b. Sounding an alarm, or
- c. Providing simple, 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).

2.4.2 Limitation on Use of Iodine

EPA policy initially developed in 1973 and reaffirmed in 1982 (memo of March 3, 1982 from J.A. Cotruvo to G.A. Jones, subject: "Policy on Iodine Disinfection") is that iodine disinfection is acceptable for short-term or limited or emergency use but that it is not recommended for long-term or routine community water supply application where iodine-containing species may remain in the drinking water.

TABLE 1

MICROBIOLOGICAL REDUCTION REQUIREMENTS

Klebsiella terrigena, a common coliform, was selected as the challenge organism to represent the coliform group. Poliovirus 1 (LSc) and rotavirus (Wa or SA-11) are common environmental viruses and show resistance to different treatment processes, thereby providing good challenges for the virus group. Giardia was selected as the cyst challenge representative because of its widespread disease impact and its resistance to chemical disinfection. The use of 4-6 micron particles or beads for testing the occlusion filtration of cysts has been demonstrated to be an accurate and practical substitute for the use of live cyst challenges. It is included as an option where disinfection or other active processes are not involved.

<u>Organism</u>	<u>Influent Challenge*</u>	<u>Minimum Required Reduction-Log</u>	
Bacteria:			
<u>Klebsiella terrigena</u> (ATCC-33257)	10 ⁷ /100 ml	6	99.9999
Virus:			
a. Poliovirus 1 (LSc) (ATCC-VR-59) and,	1 x 10 ⁷ /L	4	99.99**
b. Rotavirus (Wa or SA-11) (ATCC-VR-899 or VR-2018)	1 x 10 ⁷ /L		
Cyst (Protozoan): <u>Giardia</u>***			
a. <u>Giardia muris</u> or <u>Giardia lamblia</u> or	10 ⁶ /L	3	99.9
b. As an option for units or components based on occlusion filtration: particles or spheres, 4-6 microns	10 ⁷ /L	3	99.9

[Testing according to National Sanitation Foundation Standard 53 for cyst reduction will be acceptable]

* The influent 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.

** Virus types are to be mixed in roughly equal 1 x 10⁷/L concentrations and a joint 4 log reduction will be acceptable.

***It should be noted that new data and information with respect to cysts (i.e., Cryptosporidium or others) may in the future necessitate a review of the organism of choice and of the challenge and reduction requirements.

SECTION 3. MICROBIOLOGICAL WATER PURIFIER TEST PROCEDURES

3.1 Purpose

These tests are performed on ceramic filtration candles or units, halogenated resins and units and ultraviolet (UV) units in order to substantiate their microbiological removal capabilities over the effective use life of the purifier as defined in Table 1 and, where a pesticidal chemical is used, to determine that said chemical is not present in the effluent at excessive levels (see Section 3.5.3.4).

3.2 Apparatus

Three production units of a type are to be tested, simultaneously, if feasible; otherwise, in a manner as similar to that as possible.

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" (p. A-2 of Standard Number 53 "Drinking Water Treatment Units -- Health Effects," National Sanitation Foundation). Otherwise, the test rig must be designed to simulate field use conditions (worst case) for the unit to be tested.

3.3 Test Waters -- Non-Microbiological Parameters

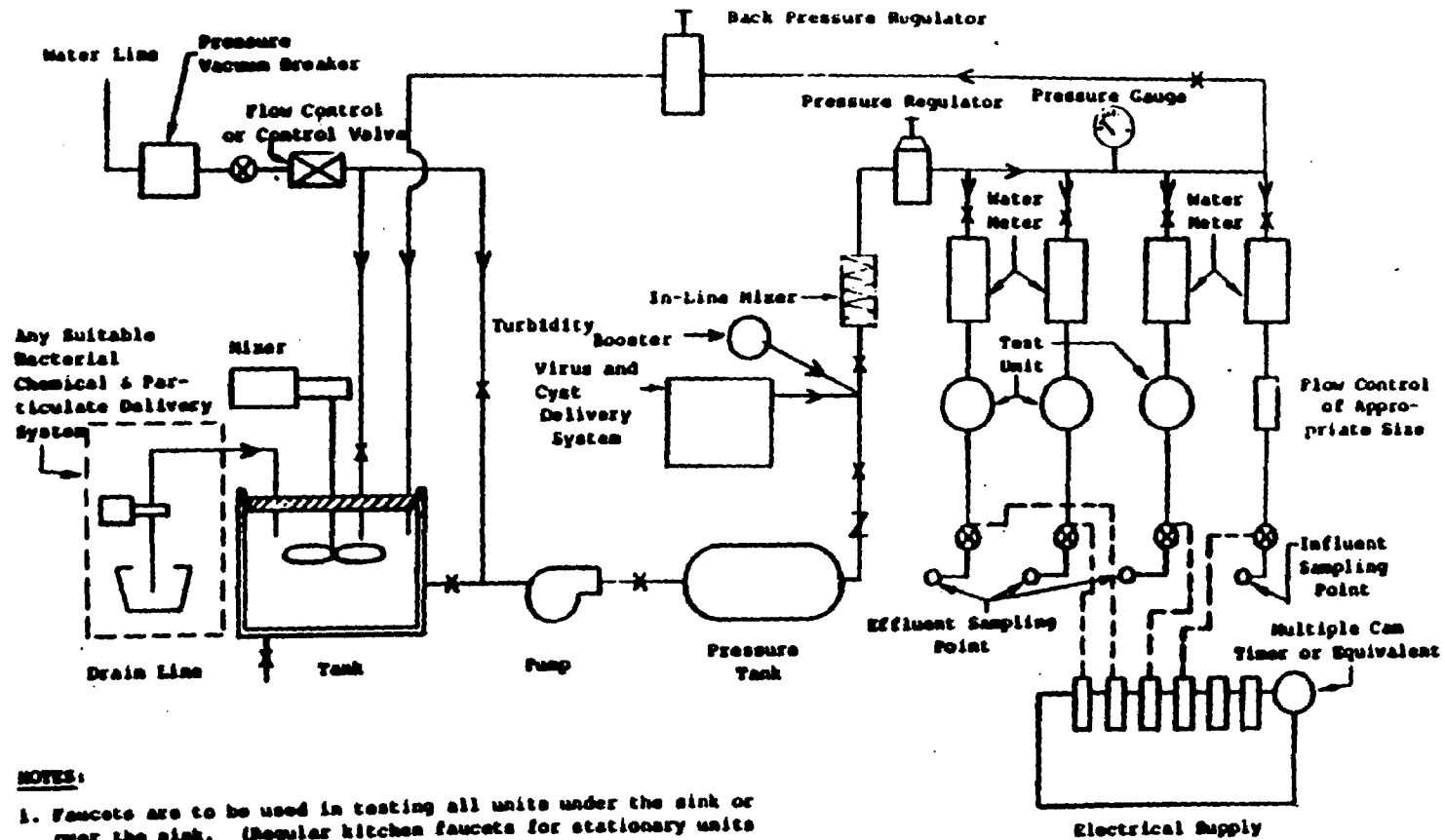
In addition to the microbiological influent challenges, the various test waters will be constituted with chemical and physical characteristics as follows:

3.3.1 Test Water #1 (General Test Water)

This water is intended for the normal non-stressed (non-challenge) phase of testing for all units and shall have specific characteristics which may easily be obtained by the adjustment of many public system tap waters, as follows:

- (a) It shall be free of any chlorine or other disinfectant residual;
- (b) pH -- 6.5 - 8.5;
- (c) Total Organic Carbon (TOC) 0.1 - 5.0 mg/L;
- (d) Turbidity 0.1 - 5 NTU;
- (e) Temperature 20°C \pm 5°C; and
- (f) Total Dissolved Solids (TDS) 50 - 500 mg/L.

FIGURE 1: TEST APPARATUS — SCHEMATIC



NOTES:

1. Faucets are to be used in testing all units under the sink or over the sink. (Regular kitchen faucets for stationary units and faucet attached units and smaller third faucets for bypass units.)
2. Faucet attached units and portable units are to be placed after the solenoid.
3. Whole house or similar large units need not use faucets. Flow can be regulated with valves placed on the effluent side.
4. All materials of construction must be suitable for use with drinking water.

- X = Shut Off Valves (not to be used for regulating flow)
- ⊗ = Solenoid Valve
- Z = Check Valve

3.3.2 Test Water #2 (Challenge Test Water/Halogen Disinfection)

This water is intended for the stressed challenge phase of testing where units involve halogen disinfectants (halogen resins or other units) and shall have the following specific characteristics:

- (a) Free of chlorine or other disinfectant residual;
- (b) (1) pH $9.0 \pm .2$, and
(2) for iodine-based units a pH of $5.0 \pm .2$ [current information indicates that the low pH will be the most severe test for virus reduction by iodine disinfection];
- (c) Total Organic Carbon (TOC) not less than 10 mg/L;
- (d) Turbidity not less than 30 NTU;
- (e) Temperature $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$; and
- (f) Total Dissolved Solids (TDS) $1,500 \text{ mg/L} \pm 150 \text{ mg/L}$.

3.3.3 Test Water #3 (Challenge Test Water/Ceramic Candle or Units With or Without Silver Impregnation)

This water is intended for the stressed challenge phase of testing for the indicated units but not for such units when impregnated with a halogen disinfectant (for the latter, use Test Water #2). It shall have the following specific characteristics:

- (a) It shall be free of any chlorine or other disinfectant residual;
- (b) pH $9.0 \pm .2$;
- (c) Total Organic Carbon (TOC) -- not less than 10 mg/L;
- (d) Turbidity -- not less than 30 NTU;
- (e) Temperature $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$; and
- (f) Total Dissolved Solids (TDS) -- $1,500 \text{ mg/L} \pm 150 \text{ mg/L}$.

3.3.4 Test Water #4 (Challenge Test Water for Ultraviolet Units)

This water is intended for the stressed phase of testing for UV units and shall have the following specific characteristics:

- (a) Free of chlorine or other disinfectant residual;
- (b) pH 6.5 - 8.5;
- (c) Total Organic Carbon (TOC) -- not less than 10 mg/L;
- (d) Turbidity -- not less than 30 NTU;

- (e) Temperature $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$;
- (f) Total Dissolved Solids (TDS) -- $1,500 \text{ mg/L} \pm 150 \text{ mg/L}$;
- (g) Color U.V. absorption (absorption at 254 nm) -- Sufficient para-hydroxybenzoic acid (PHBH) to be just below the trigger point of the warning alarm on the U.V. unit. [Note that Section 3.5.1.1.1 provides an alternative of adjusting the U.V. lamp electronically, especially when the U.V. lamp is preceded by activated carbon treatment.]

3.3.5 Test Water #5 (Leaching Test Water for Units Containing Silver)

This water is intended for stressed leaching tests of units containing silver to assure that excess levels of silver will not be leached into the drinking water. It shall have the following specific characteristics:

- (a) Free of chlorine or other disinfectant residual;
- (b) pH -- 5.0 ± 0.2 ;
- (c) Total Organic Carbon (TOC) -- approximately 1.0 mg/L ;
- (d) Turbidity -- $0.1 - 5 \text{ NTU}$;
- (e) Temperature -- $20^{\circ}\text{C} \pm 5^{\circ}\text{C}$; and
- (f) Total Dissolved Solids (TDS) -- $25 - 100 \text{ mg/L}$.

3.3.6 Recommended Materials for Adjusting Test Water Characteristics

- (a) pH: inorganic acids or bases (i.e., HCl, NaOH);
- (b) Total Organic Carbon (TOC): humic acids;
- (c) Turbidity: A.C. Fine Test Dust (Part No. 1543094)
 - from: A.C. Spark Plug Division
 - General Motors Corporation
 - 1300 North Dort Highway
 - Flint, Michigan 48556;
- (d) Total Dissolved Solids (TDS): sea salts, Sigma Chemical Co., S9883 (St. Louis, MO) or another equivalent source of TDS;
- (e) Color U.V. Absorption: p-hydroxybenzoic acid (grade: general purpose reagent).

3.4 Analytical Methods

3.4.1 Microbiological Methods

Methods in this section are considered "state-of-the-art" at the time of its preparation and subsequent improvements should be expected. Methods used for microbiological analyses should be compatible with and equal to or better than those given below.

3.4.1.1 Bacterial Tests:

- a. Chosen Organism: Klebsiella terrigena (ATCC-33257).
- b. Method of Production: The test organism will be prepared by overnight growth in nutrient broth or equivalent to obtain the organism in the stationary growth phase [Reference: Asburg, E.D., 1983, Methods of Testing Sanitizers and Bacteriostatic Substances; in Disinfection, Sterilization and Preservation (Seymour S. Block, ed.), pp. 964-980]. The organism will be collected by centrifugation and washed three times in phosphate buffered saline before use. Alternatively, the organisms may be grown overnight on nutrient agar slants or equivalent and washed from the slants with phosphate buffered saline. The suspensions should be filtered through sterile Whatman Number 2 filter paper (or equivalent) to remove any bacterial clumps. New batches of organisms must be prepared daily for use in challenge testing.
- c. State of Organism: Organisms in the stationary growth phase and suspended in phosphate buffered saline will be used.
- d. Assay Techniques: Assay may be by the spread plate, pour plate or membrane filter technique on nutrient agar, M.F.C. or a-Endo medium (Standard Methods for the Examination of Water and Wastewater, 16th edition, 1985, APHA). Each sample dilution will be assayed in triplicate.

3.4.1.2 Virus Tests:

- a. Chosen Organisms: Poliovirus type 1 (LSc) (ATCC-VR-59), and Rotavirus Strain SA-11 (ATCC-VR-899) or WA (ATCC-VR-2018).
- b. Method of Production: All stocks should be grown by a method described by Smith and Gerba (1982, in Methods in Environmental Virology, pp. 15-47) and purified by the procedure of Sharp, et al. (1975, Appl. Microbiol., 29:94-101), or similar procedure (Berman and Hoff, 1984, Appl. Environ. Microbiol., 48:317-323), as these methods will produce largely monodispersed virion particles.
- c. State of the Organism: Preparation procedure will largely produce monodispersed particles.

- d. Assay Techniques: Poliovirus type 1 may be grown in the BGM, MA-104 or other cell line which will support the growth of this virus. 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.

3.4.1.3 Cyst Tests:

- a. Chosen Organism:
- (1) Giardia lamblia or the related organism, Giardia muris, may be used as the challenge cyst.
 - (2) Where filtration is involved, tests with 4-6 micron spheres or particles have been found to be satisfactory and may be used as a substitute for tests of occlusion using live organisms (see Table 1). Spheres or particles may only be used to evaluate filtration efficacy. Disinfection efficacy can only be evaluated with the use of viable Giardia cysts.
- b. Method of Production: Giardia muris may be produced in laboratory mice and Giardia lamblia may be produced in Mongolian gerbils; inactivation results based on excystation measurements correlate well with animal infectivity results.
- c. State of the Organism: Organisms may be separated from fecal material by the procedure described by Sauch (1984, Appl. Environ. Microbiol., 48:454-455) or by the procedure described by Bingham, et al. (1979, Exp. Parasitol., 47:284-291).
- d. Assay Techniques: Cysts are first reconcentrated (500 μ l., minimum sample size) according to the method of Rice, Hoff and Schaefer (1982, Appl. Environ. Microbiol., 43:250-251). The excystation method described by Schaefer, et al. (1984, Trans., Royal Soc. of Trop. Med. & Hyg. 78:795-800) shall be used to evaluate Giardia muris cyst viability. For Giardia lamblia cysts, the excystation method described by Bingham and Meyer (1979, Nature, 277:301-302) or Rice and Schaefer (1981, J. Clin. Microbiol., 14:709-710) shall be used. Cyst viability may also be determined by an assay method involving the counting of trophozoites as well as intact cysts (Bingham, et al., 1979, Exp. Parasitol., 47:284-291).

3.4.2 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, 16th Edition, American Public Health Association, or equivalent.

3.5 Test Procedures

3.5.1 Procedure - Plumbed-in Units

- a. (1) Install three production units of a type as shown in Figure 1 and condition each unit prior to the start of the test in accordance with the manufacturer's instructions with the test water without the addition of the test contaminant. Measure the flow rate through each unit. The unit shall be tested at the maximum system pressure of 60 psig static and flow rate will not be artificially controlled.
- (2) Test waters shall have the defined characteristics continuously except for test waters 2, 3 and 4 with respect to turbidity. The background non-sampling turbidity level will be maintained at 0.1-5 NTU but the turbidity shall be increased to the challenge level of not less than 30 NTU in the following manner:
 - in the "on" period(s) prior to the sampling "on" period.
 - in the sampling "on" period when the sample actually will be taken. [Note: at least 10 unit void volumes of the 30 NTU water shall pass through the unit prior to actual sampling so as to provide adequate seasoning and uniformity before sample collection.]
- b. (1) Use appropriate techniques of dilution and insure continual mixing to prepare a challenge solution containing the bacterial contaminant. Then spike test water continuously with the influent concentration specified in Table 1.
- (2) Use appropriate techniques to prepare concentrated virus and Giardia suspensions. Feed these suspensions into the influent stream so as to achieve the influent concentrations specified in Table 1 in the following manner:
 - in the "on" period(s) prior to the sampling "on" period.
 - in the sampling "on" period when the sample actually will be taken. [Note: 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.]

- c. Purge the system of the uncontaminated water with a sufficient flow of contaminated test water. 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 contaminated 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 100% of estimated volume capacity for halogenated resins or units and for 10-1/2 days for ceramic candles or units and for U.V. units.
- d. Sampling: Samples of influent and effluent water at the specified sampling points shall be collected as shown below for the various units; these are minimum sampling plans which may be increased in number by the investigator. All samples shall be collected in duplicate from the flowing 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) and represent worst case challenge conditions. Effluent samples shall usually 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" periods, 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.)

1(a). Sampling Plan: Halogenated Resins or Units (Non-iodine Based)

Test Point (% of Estimated Capacity)	Test Water	Tests		
		Influent Background	Active Agent/ Residual	Microbiological
Start	General	X	X	X
25%			X	X
50%			X	X
After 48 hours stagnation			X	X
60%	Chal- lenge pH - 9.0 ± 0.2		X	X
75%			X	X
After 48 hours stagnation			X	X
100%			X	X

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

Test Point (% of Estimated Capacity)	Test Water	Tests		
		Influent Background	Active Agent/ Residual	Microbiological
Start	General	X	X	X
25%			X	X
50%			X	X
After 48 hours stagnation			X	X
60%	Chal- lenger pH - 9.0 ± 0.2		X	X
75%			X	X
After 48 hours stagnation			X	X
90%	Chal- lenger pH - 5.0 ± 0.2		X	X
100%			X	X
After 48 hours stagnation			X	X

2. Sampling Plan: Ceramic Candles or Units and U.V. Units

Test Point	Test Water	Tests	
		Influent Background	Microbiological
Start	General	X	X
Day 3 (middle)			X
Day 6 (middle)			X
After 48 hours stagnation			X
Day 7 (middle)	Chal- lenger		X
Day 8 (near end)			X
After 48 hours stagnation			X
Day 10-1/2			X

[Note: All days are "running days" and exclude stagnation periods. When the units contain silver, a leaching test shall be conducted as shown in Section 3.5.1.e and silver residual will be measured at each microbiological sampling point.]

- e. **Leaching Tests for Silverized Units:** Where the unit contains silver, additional tests utilizing Test Water #5 will be conducted as follows:

Test Point	Tests	
	Influent Background	Silver/Residual
Start	X	X
Day 2		X
After 48 hours stagnation		X

- f. **Alternate Sampling Plans:**

1. Since some laboratories may find it inconvenient to test some units on a 16 hour on/8 hour off cycle, two alternates are recognized:
 - go to a shorter operational day but lengthen the days of test proportionally
 - use up to 20 percent "on"/80 percent "off" for a proportionally shorter operational day
2. Sampling points must be appropriately adjusted in any alternate sampling plan.

- g. **Application of Test Waters:**

The application of test waters is designed to provide information on performance under both normal and stressed conditions; it should be the same or equivalent to the following:

1. (a) **Halogenated Resins or Units (Non-iodine based) --**
 - First 50% of test period: Test Water 1 (General)
 - Last 50% of test period: Test Water 2 (Challenge)
(pH - 9.0 ± 0.2)
- (b) **Iodinated Resins or Units --**
 - First 50% of test period: Test Water 1 (General)
 - Next 25% of test period: Test Water 2 (Challenge)
(pH - 9.0 ± 0.2)
 - Last 25% of test period: Test Water 2 (Challenge)
(but with pH - 5.0 ± 0.2)
2. **Ceramic Candles or Units --**
 - First 6 days of testing: Test Water 1 (General)
 - Last 4-1/2 days of testing: Test Water 3 (Challenge)

3. Ultraviolet (U.V.) Units --

First 6 days of testing: Test Water 1 (General)
Last 4-1/2 days of testing: Test Water 4 (Challenge)

h. Analyses and Monitoring:

1. Microbiological sampling and analysis shall be conducted of the specified influent and effluent sampling points during each indicated sampling period.
 2. Test Water Monitoring: The specified parameters of the various test waters (see Section 3.3) will be measured and recorded at each microbiological sampling point; the specified parameters will be measured at least once on non-sampling days when the units are being operated.
 3. Background chemical analyses of influent water shall be conducted at least once at the start of each test period to determine the concentration of the U.S. EPA primary inorganic contaminants, secondary contaminants and routine water parameters, not otherwise covered in the described test waters.
 4. In addition, quality assurance testing shall be conducted for the seed bacteria under environmental conditions on the first and last days of testing to make sure that there is no significant change over the test day. Populations will be measured (for example, as dispersed in the supply tank) at the beginning and end of the test day to detect possible incidental effects such as proliferation, die-off, adsorption to surfaces, etc. Relatively stable bacterial seed populations are essential to an acceptable test program.
 5. 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. Silver will additionally be measured three times in the effluent as specified in Section 3.5.1.e.
- i. Neutralization of Disinfection Activity: Immediately after collection, each test sample must be treated to neutralize any residual disinfectant. For halogen- and silver-based disinfectants this may be done by addition of thioglycollate-thiosulfate neutralizer solution (Chambers, et al., J. Amer. Water Works Assoc., 54:208-216, 1962). This solution should be prepared daily. All results are invalid unless samples are neutralized immediately upon collection.
- j. Special Provisions for Ceramic Candles or Units:
1. 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 3.5.1.d.2. shall be maintained.

2. **Cleaning of ceramic units:** Units should be cleaned according to manufacturer's directions. 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.

- k. **Halogenated units or U.V. 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.)

- l. **Special Provisions for Ultraviolet (U.V.) Units:**
 1. The units will be adequately challenged by the prescribed test waters; consequently they will be operated at normal intensity. However, where the U.V. treatment component is preceded by activated carbon treatment, the output of the U.V. lamp shall be adjusted electronically, such as by reducing the current to the lamp or other appropriate means, to be just above the alarm point. This option shall be available for use under other U.V. configurations, at the choice of the persons responsible for testing, as an alternative to the use of the U.V. absorbent, p-hydroxybenzoic acid.
 2. **Fail/safe:** Units will provide and will be tested for fail/safe warnings in the event of water quality changes or equipment failures which may interfere with its microbiological purification function.
 3. **Cleaning:** Manufacturer's guidance with respect to cleaning will be followed.

3.5.2 Procedure: Non-Plumbed Units

- a. **General:** The basic procedures given in Section 3.5.1 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.

- b. 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 to be served per day. In some cases variable flow or other non-standard conditions may be necessary to reflect a worst-case test.

3.5.3 Acceptance and Records

- 3.5.3.1 To qualify as a microbiological water purifier, three production units of a type must continuously meet or exceed the reduction requirements of Table 1, within allowable measurement tolerances for not more than ten percent of influent/effluent sample pairs, defined as follows:

Virus: one order of magnitude
Bacteria: one order of magnitude
Cysts: one/half order of magnitude

The geometric mean of all microbiological reductions must meet or exceed the requirements of Table 1. An example is given as follows:

- Unit: iodinated resin.
- Number of sample pairs over the completed test program:
10 per unit -- 3 units = 30.
- Number of allowable sample pairs where log reduction is insufficient: 10% of 30 = 3 sample pairs.
- Allowable minimum log reductions in these 3 pairs:
 - Bacteria - 5 log
 - Virus - 3 log
 - Cyst - 2-1/2 log
- Conclusion: If the geometric mean of all reductions meets or exceeds the requirements of Table 1, the indicated insufficient sample pairs will be allowed.

- 3.5.3.2 Records: All pertinent procedures and data shall be recorded in a standard format and retained for possible review until the report of results has been completely accepted by review authorities, in no case for less than a year.

- 3.5.3.3 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.

3.5.3.4 Where silver or some other chemical is used in the unit, concentrations in the effluent water must meet any National Primary Drinking Water Maximum Contaminant Level (MCL), additional Federal guidelines, or otherwise not constitute a threat to health where no MCL exists.

APPENDIX A

SUMMARY FOR BASIS OF STANDARDS AND TEST WATER PARAMETERS

A. Microbiological Reduction Requirements

1. Bacteria

Current standards for the microbiological safety of drinking water are based on the presence of coliform bacteria of which Klebsiella is a member. Members of the genus Klebsiella are also potential pathogens of man (Vlassof, 1977). Klebsiella terrigena is designated as the test organism since it is commonly found in surface waters (Izard, et al., 1981).

Experience with the use of coliform bacteria to estimate the presence of enteric bacterial pathogens in drinking water as performed over the last 75 years indicates a high degree of reliability. Required testing of more than one bacterial pathogen appears unjustified since viral and Giardia testing will be required. Enteric viruses and Giardia are known to be more resistant to common disinfectants than enteric bacterial pathogens and viruses are more resistant to removal by treatments such as filtration. Thus, any treatment which would give a good removal of both virus and Giardia pathogens would most likely reduce enteric bacteria below levels considered infectious (Jarroll, et al., 1981; Liu, et al., 1971).

The concentration of coliform bacteria in raw sewage is approximately $10^9/100$ ml. Concentrations in polluted stream waters have been found to exceed 10^5 per 100 ml (Culp, et al., 1978, Table 10).

Based on the over $10^5/100$ ml concentrations observed in highly polluted stream water and a target effluent concentration of less than $1/100$ ml, a 6 log reduction is recommended.

2. Virus

In the United States concentrations of enteroviruses are estimated to range from 10^3 - 10^4 /liter in raw sewage (Farrar and Schaub, 1971). Based on this observation it is estimated that natural waters contaminated with raw sewage may contain from 10^1 to 10^2 enteric viruses per liter.

There are currently no standards for viruses in drinking water in the United States. However, EPA has proposed a non-enforceable health-based recommended maximum contaminant level (RMCL) of zero for viruses (EPA, 1985). Several individuals and organizations have developed guidelines for the presence of viruses in drinking water and various experts have proposed standards (WHO, 1979, 1984; Berg, 1971; Melnick, 1976). It has

generally been felt that drinking water should be free of infectious virus since even one virus is potentially infectious and suggested standards are largely based on technological limits of our detection methodology. Guidelines suggested by the World Health Organization (1984) and others recommend that volumes to be tested be in the order of 100-1,000 liters and that viruses be absent in these volumes.

Assuming a target effluent level of less than one virus in 100 liters of water and a concentration of 10^4 enteric viruses in 100 liters of sewage-contaminated waters, the water purifier units should achieve at least 4 logs of virus removal.

The relative resistance of enteric viruses to different disinfectants varies greatly among the enteric viruses and even among members of the same group (i.e., enteroviruses). For example, while f2 coliphage is one of the most resistant viruses to inactivation by chlorine it is one of the most susceptible to inactivation by ozone (Harakeh and Butler, 1984). Ionic conditions and pH can also affect the relative resistance of different viruses to a disinfectant (Engelbrecht, et al., 1980). On this basis it is felt that more than one enteric virus should be tested to ensure the efficacy of any disinfection system. Poliovirus type 1 (Strain LSc) was chosen as one of the test viruses because it has been extensively used in disinfection and environmental studies as representative of the enterovirus family. It is recognized that it is not the most resistant virus to inactivation to chlorine, but is still resistant enough to serve as a useful indicator. Rotavirus is selected as the second test enteric virus since it represents another group of enteric viruses in nucleic acid composition and size. It is also a major cause of viral gastroenteritis and has been documented as a cause of waterborne gastroenteritis (Gerba, et al., 1985). The human rotavirus or the similar Simian rotavirus may be used in the test procedure. A net 4-log reduction for a joint challenge of $1 \times 10^7/L$ each for poliovirus and rotavirus is recommended.

3. Cysts (Protozoan)

Over the past several years, giardiasis has consistently been one of the most frequently reported waterborne diseases transmitted by drinking water in the United States (Craun, 1984). EPA has proposed a RMCL of zero for Giardia (EPA, 1985). Its occurrence has generally been associated with treatment deficiencies including either inadequate or no filtration. Giardiasis has not been known to occur from drinking water produced by well-operated filtration treatment plants. De Walle, et al. (1984), in a study of filtration treatment plant efficiencies, cited percent removals for Giardia in pilot plant tests as follows:

- rapid filtration with coagulation-sedimentation: 96.6-99.9%;
- direct filtration with coagulation: 95.9-99.9%.

From this research and from the lack of Giardia cases in systems where adequate filtration exists, a 3-log (99.9%) reduction requirement is considered to be conservative and to provide a comparable level of protection for water purifiers to a well-operated filtration treatment plant.

Data on environmental levels for cysts in natural waters is limited because of the difficulties of sampling and analysis. Unpublished data indicate very low levels from less than 1/L to less than 10/L. Here a 3-log reduction would provide an effluent of less than 1/100 L, comparable to the recommended virus reduction requirements.

Either Giardia lamblia or the related organism, Giardia muris, which is reported to be a satisfactory test organism (Hoff, et al., 1985), may be used as the challenge organism. Tests will be conducted with a challenge of 10^6 organisms per liter for a 3-log reduction.

Where the treatment unit or component for cysts is based on the principle of occlusion filtration alone, testing for a 3-log reduction of 4-6 micron particles or spheres (National Sanitation Foundation Standard 53, as an example) is acceptable. Difficulties in the cyst production and measurement technologies by lesser-equipped laboratories may require the use of such alternative tests where applicable.

B. Microbiological Purifier Test Procedures

1. Test Waters

a. The general test water (test water #1) is designed for the normal, non-stressed phase of testing with characteristics that may easily be obtained by the adjustment of many public system tap waters.

b. Test water #2 is intended for the stressed phase of testing where units involve halogen disinfectants.

1) Since the disinfection activity of some halogens falls with a rising pH, it is important to stress test at an elevated pH. The recommended level of 9.0 ± 0.2 , while exceeding the recommended secondary level (Environmental Protection Agency, 1984) is still within a range seen in some natural waters (Environmental Protection Agency, 1976). However, for iodine-based units, a second stressful condition is provided -- a pH of 5.0 ± 0.2 since current information indicates that the disinfection activity of iodine falls with a low pH (National Research Council, 1980). While beneath the recommended secondary level (Environmental Protection Agency, 1984) a pH of 5.0 is not unusual in natural waters (Environmental Protection Agency, 1976).

- 2) Organic matter as total organic carbon (TOC) is known to interfere with halogen disinfection. While this TOC is higher than levels in many natural waters, the designated concentration of 10 mg/L is cited as typical in stream waters (Culp/Wesner/Culp, 1978).
 - 3) High concentrations of turbidity can shield microorganisms and interfere with disinfection. While the recommended level of not less than 30 NTU is in the range of turbidities seen in secondary wastewater effluents, this level is also found in many surface waters, especially during periods of heavy rainfall and snow melt (Culp/Wesner/Culp, 1978).
 - 4) Studies with Giardia cysts have shown decreasing halogen disinfection activity with lower temperatures (Jarroll, et al., 1980); 4° C, a common low temperature in many natural waters, is recommended for the stress test.
 - 5) The amount of dissolved solids (TDS) may impact the disinfection effectiveness of units that rely on displaceable or exchange elements by displacement of halogens or resins, or it may interfere with adsorptive processes. While TDS levels of 10,000 mg/L are considered unusable for drinking, many supplies with over 2,000 mg/L are used for potable purposes (Environmental Protection Agency, 1984) The recommended level of 1,500 mg/L represents a realistic stress challenge.
- c. Test water #3 is intended for the stressed phase of testing of ceramic filtration candles or units with or without silver impregnation.
- 1) Since viruses are typically eluted from adsorbing media at high pHs (Environmental Protection Agency, 1978) it may be concluded that a high pH will provide the most stressful testing for a ceramic-type unit; consequently, the high natural water pH of 9.0 is recommended.
 - 2) Expert opinion also holds that organic material will interfere with adsorption of viruses. Thus, a high total organic carbon level of not less than 10 mg/L is recommended.
 - 3) Turbidity may enhance the entrapment and removal of microorganisms but it also may stimulate "short-circuiting" through some units. A turbidity level of 30 NTU will provide stress at time of sampling but the non-sampling level of 0.1-5 NTU will allow routine operation of units.
 - 4) Expert opinion was that low water temperatures and high TDS would most likely interfere with virus reduction by adsorption; consequently, a 4°C temperature and 1,500 mg/L TDS are recommended.

- d. Test water #4 is intended for the stressed phase of testing for ultraviolet (UV) units.
- 1) In general, high TOC, turbidity and TDS and low temperature are considered most stressful for UV, and the indicated challenge levels are the same as for test water #2.
 - 2) The pH is not critical and may range from 6.5 to 8.5.
 - 3) In order to test the UV units at their most vulnerable stage of operation, a color challenge (light absorption at 254 nm) is to be maintained at a level where UV light intensity is just above the unit's low intensity warning alarm point. However, an alternate to the absorption challenge is provided through adjusting the light intensity output of the UV lamp electronically by reducing current to the lamp, or other appropriate means, to be just above the alarm point; this approach would be particularly necessary where the UV lamp is preceded by activated carbon treatment.
- e. Test water #5 is intended for the stressed leaching tests of units containing silver. Low pH, TOC, turbidity, and TDS and higher temperature are felt to be the characteristics associated with increased leachability. The recommended pH of $5.0 \pm .2$, while being beneath the recommended secondary range of 6.5-8.5 (Environmental Protection Agency, 1984) is still found in some natural waters.

2. Test Procedures

The plan for testing and sampling is designed to reveal unit performance under both "normal" and "stressed" operating conditions. The stressed phase would utilize a set of water quality and operating conditions to give the units a realistic worst case challenge. Testing plans for a specific model might involve modifications to the recommended plan; more samples could be taken and analyzed; more units could be studied. The principle of demonstrating adequate performance even under realistic worst case conditions should be maintained and the final selected test procedures should be agreed as between investigators and reviewers or regulators.

While some aspects of the testing procedures have been utilized in actual experiments, the proposed protocol has not been verified or utilized for the various units that may be considered. Consequently, investigators and users of this protocol may find reasons to alter some aspects through their practical experience; needed changes should be discussed and cleared with involved reviewers/regulators.

APPENDIX A REFERENCES:

- Berg, G. 1971. Integrated approach to the problem of viruses in water. *J. ASCE, Sanit. Eng. Div.* 97:867-882.
- Culp/Wesner/Culp. 1978. Guidance for planning the location of water supply intakes downstream from municipal wastewater treatment facilities. EPA Report, Office of Drinking Water. Washington, DC.
- Craun, G.F. 1984. Waterborne outbreaks of giardiasis: Current status, pp. 243-261. In: D.L. Erlandsen and E.A. Meyer (eds.), Giardia and giardiasis. Plenum Press, New York.
- DeWalle, F.B., J. Engeset and W. Lawrence. 1984. Removal of Giardia lamblia cyst by drinking water treatment plants. Report No. EPA-600/52-84-069, Office of Research and Development, Cincinnati, OH.
- Engelbrecht, R.S., et al. 1980. Comparative inactivation of viruses by chlorine. *Appl. Environ. Microbiol.* 40:249-256.
- Environmental Protection Agency. 1976. Quality criteria for water. Washington, DC.
- Environmental Protection Agency. 1978. Human viruses in the aquatic environment. Report to Congress. EPA-570/9-78-006.
- Environmental Protection Agency. 1984. National secondary drinking water regulations. EPA-570/9-76-000, Washington, DC.
- Environmental Protection Agency. 1985. National primary drinking water regulations; synthetic organic chemicals, inorganic chemicals and microorganisms; Proposed rule. *Federal Register*, Nov. 13, 1985.
- Farrar, S.R., and S.A. Schaub. 1983. Viruses in wastewater sludges. In: Viral Pollution of the Environment (G. Berg, ed.). CRC Press, Boca Raton, Florida. pp. 161-163.
- Gerba, C.P., J.B. Rose and S.N. Singh. 1985. Waterborne gastroenteritis and viral hepatitis. *CRC Critical Rev. Environ. Contr.* 15:213-236.
- Harakeh, M., and M. Butler. 1984. Inactivation of human rotavirus, SA-11 and other enteric viruses in effluent by disinfectants. *J. Hyg. Camb.* 93:157-163.
- Hoff, J.C., E.W. Rice and F.W. Schaefer. 1985. Comparison of animal infectivity and excystation as measures of Giardia muris cyst inactivation by chlorine. *Appl. Environ. Microbiol.* 50:1115-1117.
- Izard, D., C. Farragut, F. Gavini, K. Kersters, J. DeLey and H. Leclerc. 1981. Klebsiella terrigena, a new species from water and soil. *Intl. J. Systematic Bacteriol.* 31:116-127.

- Jakubowski, W. 1984. Detection of Giardia cysts in drinking water. In: Giardia and Giardiasis (S.L. Erlandsen and E.A. Meyer, eds.). Plenum Press, NY. pp. 263-286.
- Jarroll, E.L., A.K. Bingham and E.A. Meyer. 1980. Giardia cyst destruction: Effectiveness of six small-quantity water disinfection methods. Am. J. Trop. Med. 29:8-11.
- Jarroll, E.L., A.K. Bingham and E.A. Meyer. 1981. Effect of chlorine on Giardia cyst viability. Appl. Environ. Microbiol. 43:483-487.
- Liu, O.C., et al. 1971. Relative resistance of 20 human enteric viruses to free chlorine in Potomac River water. Proceedings of 13th Water Quality Conference (V. Snoeyink and V. Griffin, eds.), pp. 171-195. University of Illinois.
- Melnick, J.L. 1976. Viruses in water. In: Viruses in Water (G. Berg, H.L. Bodily, E.H. Lennette, J.L. Melnick and T.G. Metcalf, eds.) Amer. Public Hlth. Assoc. Washington, DC. pp. 3-11.
- National Research Council. 1980. The disinfection of drinking water, pp. 5-137. In: Drinking Water and Health. Volume 2. Washington, DC.
- National Sanitation Foundation. 1982. Drinking water treatment units: Health effects. Standard 53. Ann Arbor, MI.
- Vlassoff, L.T. 1977. Klebsiella. In: Bacterial Indicators/Health Hazards Associated with Water (A.W. Hoadley and B.J. Dutka, eds.). American Society for Testing and Materials. Philadelphia, PA. pp. 275-288.
- World Health Organization. 1979. Human Viruses in Water, Technical Support Series 639, World Health Organization, Geneva.
- World Health Organization. 1984. Guidelines for Drinking Water Quality. Volume 1. Recommendations. World Health Organization, Geneva.

APPENDIX B

LIST OF PARTICIPANTS: TASK FORCE ON GUIDE STANDARD AND PROTOCOL FOR
TESTING MICROBIOLOGICAL WATER PURIFIERS

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APPENDIX C

RESPONSE BY REVIEW SUBCOMMITTEE* TO PUBLIC COMMENTS ON GUIDE STANDARD AND
PROTOCOL FOR TESTING MICROBIOLOGICAL WATER PURIFIERS

- A. Recommendation for the use of Giardia lamblia cysts as a replacement for Giardia muris cysts as the protozoan cyst test organisms.

Recommendation:

The subcommittee concurs with the recommendation and further endorses the use of Giardia lamblia as the preferred cyst test for evaluation of all treatment units and devices. Obviously the use of the protozoan organisms of actual health concern in testing is most desirable. Anyone finding the Giardia lamblia strain feasible for testing and cost-effective to work with is encouraged to use same instead of Giardia muris.

- B. Substitution of 4-6 micron bead or particle tests as an alternate option instead of the Giardia cysts for evaluating devices that rely strictly on occlusion filtration for microbiological removal: Several commenters criticized the use of beads or particles (e.g., A.C. fine dust) and recommended only use of live Giardia cysts for performance tests.

Discussion:

The subcommittee recognizes and favors the use of the natural human parasite, Giardia lamblia, but was not aware of any convincing scientific data which would disallow the optional use of testing with beads or particles for units or devices using only occlusion filtration to remove microorganisms. Previous development of the National Sanitation Standard (NSF) 53 (1982) requirement for cyst reduction (using 4-6 micron particles as cyst models) was based on engineering and scientific opinion and experimental evidence at that time. Specifically, Logsdon⁽¹⁾ used radioactive cyst models in the initial phase of a study of removal efficiencies for diatomaceous earth filters; subsequent experiments with Giardia muris cysts confirmed the efficacy of the diatomaceous earth filters. Further studies by Handricks⁽²⁾ and DeWalle⁽³⁾ with Giardia lamblia cysts also showed comparable reduction efficiencies for diatomaceous earth filters.

Subsequently confirmatory parallel testing results have been developed vis-a-vis 4-6 micron particles as compared to Giardia lamblia cysts. Specifically, two units listed by NSF for cyst reduction (using 4-6 micron particles)⁽⁴⁾ have also been tested and listed for 100% efficiency reduction (using Giardia lamblia cysts) by Hibler⁽⁵⁾;

*S.A. Schaub; F.A. Bell, Jr.; P. Berger; C. Gerba; J. Hoff; P. Regunathan; and R. Tobin. [Includes additional revision pursuant to Scientific Advisory Panel review (Federal Insecticide, Fungicide, and Rodenticide Act)]

- (1) Everpure Model QC4-SC
- (2) Royal Doulton Model F303.

Again we prefer the use of the human pathogen, Giardia lamblia; however, no experimental data has been provided regarding the lack of validity or of failure in previous tests utilizing beads or particles of 4-6 microns. In most cases the bacterial or viral challenges to occlusion filters will represent a greater problem in terms of microbiological reduction requirements than will cysts. Therefore, without substantiation of deficiencies, the use of 4-6 micron beads or particles is considered to be as feasible as the use of live cysts for routine performance testing of water filtration (occlusion) devices.

Recommendation:

Recommend retaining the optional use of 4-6 micron particles or beads for cyst reduction testing in occlusion filtration devices only.

References:

- (1) Logsdon, G.S., et al. Alternative Filtration Methods for Removal of Giardia Cysts and Cyst Models, JAWWA, February, 1981.
- (2) Logsdon, G.S., Hendricks, D.W., et al. Control of Giardia Cysts by Filtration: The Laboratory's Role, presented AWWA Water Quality Technology Conference, December 6, 1983.
- (3) DeWalle, et al. Removal of Giardia lamblia Cysts by Drinking Water Treatment Plants, Grant No. R806127, Report to Drinking Water Research Division, U.S. EPA (ORD/MERL), Cincinnati, Ohio.
- (4) National Sanitation Foundation, 1986, Listing of Drinking Water Treatment Units, Standard 53. May 21, 1986.
- (5) Hibler, C.P. 1984. An Evaluation of Filters in the Removal of Giardia lamblia. Water Technology, July, 1984, pp. 34-36.

- C. Alternate assay techniques for cyst tests (Jensen): Proposed alterations in cyst tests include a different method for separating cysts from fecal material and an assay method involving the counting of trophozoites as well as intact cysts. Both alterations have been used by Bingham, et al. (1979, Exp. Parasitol., 47:284-291).

Recommendation:

These alterations appear to be reasonable laboratory procedures, supported by a peer-reviewed article and will be included in the Report as options for possible development and use by interested laboratories.

- D. The use of pour plate techniques as an option for Klebsiella terrigena bacteria analyses.

Recommendation:

The pour plate technique adds a heat stress factor to the bacteria which constitutes a possible deficiency. However, it is a recognized standard method and probably will not adversely affect the Klebsiella terrigena. Consequently, it will be added to the Report as one of the acceptable techniques.

- E. Option of using Escherichia coli in lieu of Klebsiella terrigena for the bacterial tests.

Discussion:

Appendix A, Section A.1. of the Guide Standard and Protocol sets forth the basis for selection of K. terrigena as the test bacteria. The selection was made along pragmatic lines emphasizing the occurrence of K. terrigena in surface waters and that it would represent the enteric bacteria. It was also pointed out that the tests with virus and Giardia were expected to be more severe than the bacterial tests. For comprehensiveness, bacterial tests were included in the protocol but were not felt to be as crucial as the virus and Giardia tests.

E. coli, or any number of other generally accepted indicator bacteria, could be used for the test program if they were shown to have good testing and survival characteristics (equivalent to K. terrigena) by the interested research laboratory.

Recommendation:

The intent of the Guide Standard and Protocol is to provide a base-line program subject to modification when properly supported by an interested laboratory. Consequently, any laboratory could propose and with proper support (demonstrating challenge and test equivalency to K. terrigena) use Escherichia coli or one of the other enteric bacteria. This idea will be included in revised wording in Section 1.2.2, "General Guide."

- F. Performance requirements for Giardia cysts and virus in relation to the EPA-Recommended Maximum Contamination Levels (RMCLs) of zero.

Discussion:

The RMCLs of zero for Giardia and viruses which have been proposed by EPA are health goals. They are not enforceable standards since to assure the presence of "no organisms" would require an infinite sample. The rationale for the recommended performance requirements for Giardia cysts and virus is set forth in Sections A.2 and A.3 of Appendix A. We feel that these requirements together with the application of realistic worst case test conditions will provide a

conservative test for units resulting in treated effluent water equivalent to that of a public water supply meeting the microbiological requirements and intent of the National Primary Drinking Water Regulations.

Recommendation:

Retain recommended performance (log reduction) requirements for cyst and virus reduction.

- G. Rotavirus and its proposed assay: One commenter states that the rotavirus tests are impractical because Amirtharajah (1986, JAWWA, 78:3:34-49) cites "no satisfactory culture procedures available for analysis of these pathogens and, therefore, monitoring would not be feasible."

Discussion:

Section 3.4.1.2, "Virus Tests" of the Report, presents means for culturing and assaying rotaviruses. The means for doing the rotavirus tests are available and are practical for application in the laboratory. Dr. Amirtharajah was referring to the field collection, identification in the presence of a wide variety of microorganisms, and quantification as not being "satisfactory." Laboratory analysis of rotaviruses is practical but their field monitoring may not yet be feasible.

Further, the selection of both poliovirus and rotavirus as test viruses was necessitated by the fact that the surface adsorptive properties and disinfection resistance of the various enteric viruses have been shown to differ significantly by virus group and by strains of a specific virus. While all enteric viruses and their strains could not be economically tested, it was determined by the task force that at least two distinctly different virus types should be tested to achieve some idea of the diversity of removal by the various types of water purifiers. Polio and rota viruses have ~~have~~ distinctly different physical and chemical characteristics representative of the viruses of concern. Polioviruses are small single stranded RNA viruses with generally good adsorptive properties to surfaces and filter media while rotaviruses are over twice as large, are double stranded RNA and in some studies have been found to possess less potential for adsorption onto surfaces or filter media. These two viruses also have been demonstrated to have somewhat different disinfection kinetics.

Recommendation:

Retain the rotavirus test requirements.

- H. Definition of microbiological water purifier: One general comment requested redefinition based on "lack of any virus removal" requirement in the EPA primary drinking water regulations, so that no virus reduction requirement should be included. Also, it was claimed that the separation of purifiers from non-purifiers would be a "disservice to consumers and other users."

Discussion:

Virus are recognized in the EPA regulations vis-a-vis a proposed recommended maximum contaminant level of zero. Since virus monitoring for compliance with a possible MCL is not yet feasible, a treatment requirement is necessary. Virus control will be considered in the Safe Drinking Water Act filtration and disinfection treatment regulations. The reduction of viruses by treatment is discussed by Amirtharajah (1986, JAWWA, 78:3:34-49).

With respect to consumers and other users, we feel that the current definition is appropriate and necessary. The average consumer cannot be expected to know the difference between viruses, bacteria and cysts, or when a raw water will or will not contain any of these organisms. In order to protect the average consumer, the subject units either alone or with supplementary treatment, should be able to cope with all of the specified organisms.

Recommendation:

Retain the current definition for microbiological water purifier.

- I. Coverage of units: Several comments related to the coverage of units. These questions are addressed individually as follows:
1. Ultraviolet units that are used for supplemental treatment of water from public water system taps should not be covered. We agree that such units are not covered and parenthetical language has been included in Section 1.3.2.3 to clarify this point.
 2. A special status should be given to units which remove Giardia and bacteria but not virus. Specifically, the meaning of Section 1.2.4, "Exceptions," was addressed. The "Exceptions" section was specifically developed to relate to the problem of public water systems having disinfection but no filtration on a surface supply. Cysts alone have been found to survive disinfection treatment and could be present in such treated waters. In this case an effective cyst filter serves an independent, beneficial purpose and should not be required to be a microbiological water purifier. However, such a unit should not be used as sole treatment for untreated raw water. Additional parenthetical language has been added to Section 1.2.4.
 3. The entire treatment unit or system should be tested, not just a single component. We agree but believe that it is sufficiently clear without providing additional language.

4. The protocol should be expanded to cover units for the reduction of TCE, EDB and other chemical pollutants. We felt that the introduction of non-microbiological claims to the standard would make it large, unwieldy and duplicative of an existing third-party standards and testing program (see Section 1.2.5).

J. Alleged preference of National Sanitation Foundation (NSF) over other laboratories for conducting the microbiological water purifier testing protocol. The comment indicated that we were giving NSF preferential treatment "to the detriment of other laboratories well qualified to perform the required protocol."

Discussion:

We have made appropriate references to existing standards (#42 and #53) developed by the NSF standards development process. Standard 53, the health effects standard, was developed by a broadly based Drinking Water Treatment Units Committee, including representatives from local, State and Federal health and environmental agencies, universities, professional and technical associations, as well as water quality industry representatives. It was adopted in 1982 and the only test from it utilized in our Report has been substantiated as described in Part B of this "Response."

Nowhere in our report have we advocated NSF (or any other laboratory) as the prime or only laboratory for implementing "the required protocol."

Recommendation:

No action needed.

K. Instruction concerning effective lifetime. One comment described an alternate means for determining lifetime where a ceramic unit is "brushed" to renew its utility and is gradually reduced in diameter. A gauge is provided to measure diameter and to determine when replacement is needed.

Recommendation:

Where a manufacturer provides a satisfactory "other" means of determining lifetime, this should be accepted. Appropriate words have been added to Section 2.4.1.C.

L. Ceramic candles should not be cleaned during testing because some consumers would not clean them and this would provide the "worst case test." One comment asserted this point.

Discussion:

There is some truth to this proposition. However, the other approach may also have validity. Frequent brushing may reduce filtration efficiency. In any event, where a manufacturer prescribes filter cleaning and how to do it, and provides a gauge to determine lifetime, we feel the testing program is bound to follow the manufacturer's directions.

Recommendation:

No change needed.

- M. Scaling up or down. One comment points out that one or more manufacturers may vary size of treatment units by increasing or decreasing the number of operative units rather than the size of the operative unit. The comment suggests allowing scaling based on size of operative unit.

Recommendation:

We agree with the comment and have added clarifying words to Section 3.5.3.3.

- N. Turbidity level of "not less than 30 NTU" for ceramic candles or units. One comment states that "Such levels are impossible to utilize in testing mechanical filtration devices which will clog entirely or require such frequent brushing as to render the test impossible as a practical matter."

Discussion:

We recognized the potential "clogging problems" in Section 3.5.1.a(2) where the 30 NTU water is only to be applied immediately before and during each sampling event; the non-sampling turbidity level, which will be applied over 90% of the "on" time, is currently set at not less than 10 NTU.

Turbidity levels of 30 NTU are commonly found in surface waters during heavy rainfall or snow melt. Treatment units may be used under these circumstances, so this challenge level should be retained. However, most usage will occur under background conditions so the non-sampling turbidity levels should be 0.1-5 NTU.

Recommendations:

- (1) Retain sampling turbidity level of not less than 30 NTU, and
- (2) Change non-sampling turbidity level to 0.1-5 NTU. Appropriate wording changes have been introduced in Section 3.5.1.a(2) and in Appendix A, Section B.

- O. Chlorine in test water #5. One comment asserts that chlorine "tends to increase silver ion leaching activity" and that a high chlorine level should be included in the silver leaching test; but no reference or evidence, however, is provided to back this assertion.

Discussion:

We have no compelling evidence or reason to expect that chlorine will enhance the leaching of silver. However, the prescribed low pH and TDS levels will provide a clearly severe test for silver leaching.

Recommendation:

No change needed.

- P. Unnecessary difficulty and expense of test protocols. Several comments were made under this general heading. These comments are outlined and discussed as follows:
1. Too many sampling events are required; sampling of a few units at start, middle and finish should be satisfactory: The committee has carefully laid out the standard and protocol and we feel the minimum sampling plan must be maintained for the consumers' health protection.
 2. Three units are too many to study; parallel testing of two units should be satisfactory: For consumer protection, the Disinfectants Branch, Office of Pesticide Programs, has traditionally required the testing of three units. The committee recognizes the additional cost involved in testing a third unit but feels that this will provide a minimum level of assurance to prevent infectious disease and recommends retention of the 3-unit requirement.
 3. The protocol requires large tanks and microbiological reseeded on a daily basis: We feel that the tank size requirements are not extreme and can be met by an interested laboratory. With respect to reseeded, it should be pointed out that virus and cyst seeding need only be conducted immediately before and during the sampling "on" period (see Section 3.5.1.b(2)), equivalent to less than 10% of the "on" time. This "spot" seeding for viruses and cysts recognized the expense and difficulty of maintaining large populations of these organisms. Continuous seeding was provided for bacteria because they are easier to grow and maintain and might have the capacity to grow through some units, given enough time and opportunity.
 4. Challenge levels of contaminants are too high compared to known environmental conditions and the required log reductions exceed Safe Drinking Water Act requirements: As explained in a footnote to Table 1, Section 2, the influent challenges may constitute greater concentrations than would be anticipated in source

waters. These levels are necessary to test properly for the required log reductions without having to utilize sample concentration procedures which are time/labor intensive and which may, on their own, introduce quantitative errors to the microbiological assays. As mentioned in Part I of this paper, the log reductions for bacteria, virus and Giardia have been suggested for public water system treatment in a paper by Amirtharajah (1986, JAWWA, 78:3:34-49). The reductions in the microbiological purifier standard are entirely compatible with the reductions cited for public water supply treatment.