July 2006
NSF 05/12b/EPADWCTR
EPA/600/R-06/131

Environmental Technology Verification Report

Physical Removal of Microbial Contaminants in Drinking Water

Watts Premier, Inc. WP-4V Point-of-Use Drinking Water Treatment System

Prepared by

NSF International

Under a Cooperative Agreement with
U.S. Environmental Protection Agency

This page is intentionally blank

THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

NSF International

ETV Joint Verification Statement

TECHNOLOGY TYPE:	**POINT-OF-USE DRINKING WATER TREATMENT SYSTEM**
APPLICATION:	**REMOVAL OF MICROBIAL CONTAMINANTS IN DRINKING WATER**
PRODUCT NAME:	**WATTS PREMIER WP-4V**
VENDOR:	**WATTS PREMIER**
ADDRESS:	**1725 WEST WILLIAMS DR. SUITE C-20 PHOENIX, AZ 85027**
PHONE:	**800-752-5582**
INTERNET	**http://www.wattspremier.com**

NSF International (NSF) manages the Drinking Water Systems (DWS) Center under the U.S. Environmental Protection Agency's (EPA) Environmental Technology Verification (ETV) Program. The DWS Center recently evaluated the performance of the Watts Premier WP-4V point-of-use (POU) reverse osmosis (RO) drinking water treatment system. NSF performed all of the testing activities and also authored the verification report and this verification statement. The verification report contains a comprehensive description of the test.

EPA created the ETV Program to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and more cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized standards and testing organizations, stakeholder groups (consisting of buyers, vendor organizations, and permitters), and with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

ABSTRACT

The Watts Premier WP-4V four-stage POU RO system was tested for removal of bacteria and viruses at NSF's Drinking Water Treatment Systems Laboratory. Five systems were challenged with the bacteriophage viruses fr and MS2, and the bacteria *Brevundimonas diminuta*. The virus challenges were conducted at three different pH settings (6, 7.5, and 9) to assess whether pH influences the performance of the RO membrane. The bacteria challenges were conducted only at pH 7.5.

The challenge concentrations ranged from 3.8 to 5.0 logs for the viruses, and 6.4 to 7.2 logs for the bacteria. The log reductions ranged from 1.3 to 6.4 $\log_{10}$ for *B. diminuta*, with an average of 2.1 $\log_{10}$. The virus log reductions ranged from 1.4 to 3.6 $\log_{10}$ for fr, and 1.2 to 3.7 $\log_{10}$ for MS2. The average virus $\log_{10}$ reductions were 2.5 and 2.7, respectively. The virus challenge data does not indicate that the pH of the challenge water influenced removal by the RO membrane. See Table VS-2 below for the complete log reduction data.

TECHNOLOGY DESCRIPTION

The following technology description was provided by the manufacturer and has not been verified.

The WP-4V is a four-stage POU drinking water treatment system using sediment filtration, activated carbon filtration, and reverse osmosis. Treated water is stored in a three-gallon storage tank. The WP-4V is certified by NSF to NSF/ANSI Standard 58 – *Reverse Osmosis Drinking Water Treatment Systems*. It has a certified production rate of 9.06 gallons per day.

Incoming water first passes through a sediment filter to remove particulate matter, such as rust and silt, and then through a carbon filter to remove chlorine or other contaminants. The third stage of treatment is the reverse osmosis membrane, which removes a wide variety of inorganic and larger molecular weight organic contaminants, and also protozoan cysts such as Cryptosporidium and Giardia. The permeate water is sent to a 3-gallon maximum capacity storage tank. Upon leaving the storage tank, the water passes through a second carbon filter to remove organic chemicals and other taste and odor causing substances before dispensing through the faucet. The pre-membrane carbon and sediment filters were not tested, because they are only designed to remove chlorine and particulate matter to protect the RO membrane.

VERIFICATION TESTING DESCRIPTION

Test Site

The testing site was the Drinking Water Treatment Systems Laboratory at NSF in Ann Arbor, Michigan. A description of the test apparatus can be found in the test/QA plan and verification report. The testing was conducted in June and July of 2005.

Methods and Procedures

The testing methods and procedures are detailed in the Test/QA Plan for Verification Testing of the Watts Premier WP-4V Point-of-Use Drinking Water Treatment System for Removal of Microbial Contamination Agents. Five WP-4V systems were tested for bacteria and virus removal performance using the bacteriophage viruses fr and MS2, and the bacteria Brevundimonas diminuta. The challenge organisms were chosen because they are smaller than most other viruses and bacteria, and so provide a conservative estimate of performance. NSF also used a genetically engineered strain of B. diminuta. The NSF Microbiology Laboratory inserted into a culture of B. diminuta strain 19146 a gene conferring resistance to the antibiotic kanamycin. This allowed the Microbiology Laboratory to use a growth media

amended with 50 μg/mL of kanamycin to prohibit heterotrophic plate count (HPC) bacteria in the treated water samples from growing along with the kanamycin resistant B. diminuta.

Five systems were evaluated. The systems were installed on a test rig and conditioned according to the vendor's instructions (fill the storage tanks and dispensing the contents to a drain three times), and then were conditioned for another five days. Prior to testing, the systems were evaluated for reduction of total dissolved solids (TDS) to ensure that the systems undergoing testing were representative of the expected performance of the system.

The test water for the bacteria challenges was set to pH 7.5 ± 0.5, while the virus challenges were conducted at pH 6.0 ± 0.5, 7.5 ± 0.5, and 9.0 ± 0.5. The challenge schedule is shown in Table VS-1. The virus challenges were conducted at different pH settings to evaluate whether the surface charges of the viruses influenced their removal through electrostatic forces versus mechanical filtration. Viruses have different surface charges, or different strengths of negative or positive charge, depending on their isoelectric point and the pH of the water. The isoelectric point is the pH at which the virus surface is neutrally charged. MS2's isoelectric point is pH 3.9, and fr's is pH 8.9. In solutions above the isoelectric point, the virus is negatively charged. Below the isoelectric point, the virus is positively charged.

Table VS-1. Challenge Schedule

Day	Surrogate Challenge	pH
1	*B. diminuta*	7.5 ± 0.5
2	fr and MS2	6.0 ± 0.5
3	fr and MS2	7.5 ± 0.5
4	Kanamycin Resistant *B. diminuta*	7.5 ± 0.5
5	fr and MS2	9.0 ± 0.5

For each challenge, the systems were operated for one tank-fill period (approximately four to five hours). The end of this period was evident through engagement of each system's automatic shutoff mechanism, which causes the flow of reject water to cease. Influent water samples were collected at the beginning and end of each challenge period. After each system ceased operation, the contents of the product water storage tanks were emptied into sterile containers, and samples were collected for microbiological analysis. All samples were enumerated in triplicate. Following each challenge period, the systems were flushed by operating them for one tank-fill period using water without challenge organisms.

VERIFICATION OF PERFORMANCE

As discussed above, the systems were first subjected to a TDS reduction test to verify that the RO membranes would perform as expected. The observed TDS reduction ranged from 89% to 96%. The certified TDS reduction for the WP-4V is 97%.

The bacteria and virus $\log_{10}$ reduction data is presented in Table VS-2. The $\log_{10}$ reduction of *B. diminuta* ("normal" and kanamycin resistant *B. diminuta* combined) ranged from 1.3 to 6.4, with an average $\log_{10}$ reduction of 1.9. The challenge organisms were detected in the effluent samples for all test units but Unit 2 for the "normal" *B. diminuta* challenge. Since the Unit 2 effluent count for kanamycin resistant *B. diminuta* was 4.3 $\log_{10}$, and all other effluent samples had bacteria counts greater than 4 $\log_{10}$ (data not shown), it is possible that there was a sampling or analytical error associated with the Unit 2 "normal" *B. diminuta* sample. Therefore, that sample was not included in the mean $\log_{10}$ reduction calculation for the bacteria.

The virus challenge data showed similar performance. The $\log_{10}$ reduction of the fr virus ranged from 1.4 to 3.6, with an overall mean of 2.5. The $\log_{10}$ reduction of MS2 ranged from 1.2 to 3.7, with an overall mean of 2.6. A visual comparison of the $\log_{10}$ reductions versus the challenge water pH shows the mean $\log_{10}$ reductions decreasing with increasing pH. However, an examination of the 95% confidence intervals around the means (see verification report for data) shows that the decreases are not statistically significant.

The minimum observed log reductions equal removal of 95% of *B. diminuta*, and 94% of the viruses.

Table VS-2. Bacteria and Virus Log Reduction Data

Target pH	Initial Measured pH	Final Measured pH	Challenge Organisms	$\log_{10}$ Influent Challenge	Geometric Mean $\log_{10}$ Reduction					
					Unit 1	Unit 2	Unit 3	Unit 4	Unit 5	Mean
7.5 ± 0.5	7.6	7.8	*B. diminuta*	6.4	1.8	6.4*	1.3	1.5	1.6	1.5
7.5 ± 0.5	7.5	7.8	Kanamycin Resistant *B. diminuta*	7.2	1.4	2.9	2.6	2.6	3.1	2.4
6.0 ± 0.5	6.1	6.5	fr	3.9	1.8	3.1	3.6	3.4	3.0	2.9
			MS2	3.8	2.3	3.4	3.7	3.6	2.9	3.1
7.5 ± 0.5	7.5	7.7	fr	4.5	1.9	2.4	2.3	3.1	2.8	2.5
			MS2	4.2	1.7	2.4	2.4	3.4	3.2	2.5
9.0 ± 0.5	8.9	9.0	fr	5.0	1.4	2.3	2.1	2.3	2.6	2.1
			MS2	4.6	1.2	2.4	2.0	2.3	3.0	2.1
						Overall Means:			*B. diminuta*	1.9
									fr	2.5
									MS2	2.6

*Number not included in mean log reduction calculation.

QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

NSF provided technical and quality assurance oversight of the verification testing as described in the verification report, including a review of nearly 100% of the data. NSF personnel also conducted a technical systems audit during testing to ensure the testing was in compliance with the test plan. A complete description of the QA/QC procedures is provided in the verification report.

Original signed by Sally Gutierrez 08/11/06
Sally Gutierrez Date
Director
National Risk Management Research Laboratory
Office of Research and Development
United States Environmental Protection Agency

Original signed by Robert Ferguson 08/23/06
Robert Ferguson Date
Vice President
Water Systems
NSF International

NOTICE: Verifications are based on an evaluation of technology performance under specific, predetermined criteria and the appropriate quality assurance procedures. EPA and NSF make no expressed or implied warranties as to the performance of the technology and do not certify that a technology will always operate as verified. The end-user is solely responsible for complying with any and all applicable federal, state, and local requirements. Mention of corporate names, trade names, or commercial products does not constitute endorsement or recommendation for use of specific products. This report is not an NSF Certification of the specific product mentioned herein.

Availability of Supporting Documents

Copies of the test protocol, the verification statement, and the verification report (NSF report # NSF 06/12b/EPADWCTR) are available from the following sources:

(NOTE: Appendices are not included in the verification report. Appendices are available from NSF upon request.)

1. ETV Drinking Water Systems Center Manager (order hard copy)
   NSF International
   P.O. Box 130140
   Ann Arbor, Michigan 48113-0140

2. Electronic PDF copy
   NSF web site: http://www.nsf.org/etv
   EPA web site: http://www.epa.gov/etv

This page is intentionally blank

July 2006

Environmental Technology Verification Report

Physical Removal of Microbial Contaminants in Drinking Water

Watts Premier Incorporated
WP-4V Point-of-Use Drinking Water Treatment System

Prepared by:

NSF International
Ann Arbor, Michigan 48105

Under a cooperative agreement with the U.S. Environmental Protection Agency

Jeffrey Q. Adams, Project Officer
National Risk Management Research Laboratory
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

Notice

The U.S. Environmental Protection Agency (USEPA), through its Office of Research and Development, has financially supported and collaborated with NSF International (NSF) under Cooperative Agreement No. R-82833301. This verification effort was supported by the Drinking Water Systems (DWS) Center, operating under the Environmental Technology Verification (ETV) Program. This document has been peer-reviewed, reviewed by NSF and USEPA, and recommended for public release.

Foreword

The U.S. Environmental Protection Agency (USEPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, USEPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory (NRMRL) is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threaten human health and the environment. The focus of the Laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research provides solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by USEPA's Office of Research and Development to assist the user community and to link researchers with their clients.

Sally Gutierrez, Director
National Risk Management Research Laboratory

Table of Contents

Verification Statement .... VS-i
Title Page i
Notice ii
Foreword iii
Table of Contents iv
List of Tables vi
List of Figures vi
Abbreviations and Acronyms vii
Acknowlegements viii

Chapter 1 Introduction 1
1.1 Environmental Technology Verification (ETV) Program Purpose and Operation 1
1.2 Purpose of Verification 1
1.3 Development of Test/Quality Assurance (QA) Plan 1
1.3.1 Bacteria and Virus Surrogates 2
1.4 Testing Participants and Responsibilities 3
1.4.1 NSF International 3
1.4.2 Watts Premier 4
1.4.3 U.S. Environmental Protection Agency 4

Chapter 2 Equipment Description 5
2.1 RO Membrane Operation 5
2.2 Equipment Capabilities 5
2.3 System Components 5
2.4 Same or Similar Models 5
2.5 System Operation 6
2.6 Rate of Waste Production 8
2.7 Equipment Operation Limitations 8
2.8 Operation and Maintenance Requirements 8

Chapter 3 Methods and Procedures 9
3.1 Introduction 9
3.2 Verification Test Procedure 9
3.2.1 Test Rig 9
3.2.2 Test Rig Sanitization 9
3.2.3 Test Water 9
3.2.3.1 Base Water 9
3.2.3.2 Bacteria and Virus Challenges 11
3.2.4 System Operation 12
3.2.4.1 System Installation 12
3.2.4.2 TDS Reduction System Check 12
3.2.4.3 Additional Conditioning 12
3.2.4.4 Challenge Testing 12

3.3 Analytical Methods 13
3.3.1 Water Quality Analytical Methods 13
3.3.2 Microbiology Analytical Methods 14
3.3.2.1 Sample Processing, and Enumeration of Viruses 14
3.3.2.2 Bacteria Cultivation 14
3.3.2.3 Preparation of *B. diminuta* Challenge Suspensions 14
3.3.2.4 Sample Processing and Enumeration of *B. diminuta* 15

Chapter 4 Results and Discussion 16
4.1 TDS Reduction 16
4.2 Bacteria Reduction 16
4.3 Virus Reduction 17

Chapter 5 QA/QC 18
5.1 Introduction 18
5.2 Test Procedure QA/QC 18
5.3 Sample Handling 18
5.4 Chemistry Analytical Methods QA/QC 18
5.5 Microbiology Laboratory QA/QC 18
5.5.1 Growth Media Positive Controls 18
5.5.2 Negative Controls 19
5.5.3 Bacteria Cell Size 19
5.6 Documentation 19
5.7 Data Review 19
5.8 Data Quality Indicators 20
5.8.1 Representativeness 20
5.8.2 Accuracy 20
5.8.3 Precision 20
5.8.4 Completeness 21
5.8.4.1 Completeness Measurements 21
5.9 Measurements Outside of the Test/QA Plan Specifications 22

Chapter 6 References 23

Appendices

Appendix A Bacteria and Virus Counts, and Water Chemistry Data

List of Tables

Table 1-1. Virus and Host ATCC Designations 3
Table 3-1. Challenge Schedule 13
Table 4-1. TDS Reduction Test Results 16
Table 4-2. Bacteria Log Reduction Data 17
Table 4-3. Virus Log Reduction Data....................17
Table 5-1. Bacteria Cell Size Measurements....................19
Table 5-2. Completeness Requirements 21

List of Figures

Figure 2-1. Photograph of the WP-4V System....................6
Figure 2-2. Schematic Diagram of the WP-4V....................7
Figure 3-1. Schematic Diagram of Test Rig....................10
Figure 3-2. Systems Installed on Test Rig....................11

Abbreviations and Acronyms

ANSI	American National Standards Institute
ASTM	American Society of Testing Materials
ATCC	American Type Culture Collection
°C	Degrees Celsius
CFU	Colony Forming Unit
cm	Centimeter
DWS	Drinking Water Systems
ETV	Environmental Technology Verification
°F	Degrees Fahrenheit
HPC	Heterotrophic Plate Count
L	Liter
mg	Milligram
mL	Milliliter
nm	Nanometer
NRMRL	National Risk Management Research Laboratory
NSF	NSF International (formerly known as National Sanitation Foundation)
PBDW	Phosphate-Buffered Dilution Water
PFU	Plaque Forming Unit
POE	Point-of-Entry
POU	Point-of-Use
psig	Pounds per Square Inch Gauge
QA	Quality Assurance
QC	Quality Control
QA/QC	Quality Assurance/Quality Control
RO	Reverse Osmosis
RPD	Relative Percent Difference
SLB	Saline Lactose Broth
SOP	Standard Operating Procedure
TDS	Total Dissolved Solids
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
μg	Microgram
μL	Microliter
μm	Micrometer
μmho	Micromho
μS	MicroSieman
USEPA	U. S. Environmental Protection Agency

Acknowledgments

NSF International was responsible for all elements in the testing sequence, including collection of samples, calibration and verification of instruments, data collection and analysis, data management, data interpretation and the preparation of this report.

The Manufacturer of the Equipment was:

Watts Premier Incorporated
1725 West Williams Drive
Suite C-20
Phoenix, AZ 85027

NSF wishes to thank the members of the expert technical panel for their assistance with development of the test plan.

Chapter 1
Introduction

1.1 Environmental Technology Verification (ETV) Program Purpose and Operation

The U.S. Environmental Protection Agency (USEPA) has created the ETV Program to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and more cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized standards and testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; and with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders; by conducting field or laboratory testing, collecting and analyzing data; and by preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The USEPA has partnered with NSF International (NSF) under the ETV Drinking Water Systems (DWS) Center to verify performance of drinking water treatment systems that benefit the public and small communities. It is important to note that verification of the equipment does not mean the equipment is “certified” by NSF or “accepted” by USEPA. Rather, it recognizes that the performance of the equipment has been determined and verified by these organizations under conditions specified in ETV protocols and test plans.

1.2 Purpose of Verification

The purpose of this verification was to evaluate treatment system performance under a simulated intentional or non-intentional microbiological contamination event. Because any contamination event would likely be short-lived, the challenge period for each chemical lasted only a few hours. Long-term performance over the life of the membrane was not investigated.

1.3 Development of Test/Quality Assurance (QA) Plan

USEPA’s “Water Security Research and Technical Support Action Plan” (USEPA, 2004) identifies the need to evaluate point-of-use (POU) and point-of-entry (POE) treatment system capabilities for removing likely contaminants from drinking water. As part of the ETV program NSF developed a test/QA plan for evaluating POU reverse osmosis (RO) drinking water treatment systems for removal of microbial contaminants. To assist in this endeavor, NSF

assembled an expert technical panel, which gave suggestions on a protocol design prior to development of the test/QA plan. Panel members included experts from USEPA, United States Army, and United States Centers for Disease Control and Prevention, Division of Parasitic Diseases, as well as a water utility microbiologist, a university professor, and an independent consultant in the POU drinking water treatment systems industry.

The product-specific test/QA plan for evaluating the WP-4V was entitled *Test/QA Plan for Verification Testing of the Watts Premier WP-4V Point-of-Use Drinking Water Treatment System for Removal of Microbial Contamination Agents.* This test/QA plan uses surrogate bacteria and viruses in place of testing with the actual agents of concern. The test organisms serve as surrogates not only for bacteria and viruses, but also protozoa, such as *Cryptosporidium* oocysts. Please note that this test plan does not cover chemical agents derived from microorganisms, such as ricin or botulism toxin.

By participating in this ETV, Watts Premier has obtained USEPA and NSF verified independent test data indicating potential user protection against intentional or unintentional biological contamination of drinking water. POU RO systems are not typically marketed as microbiological water purifiers that remove bacteria and viruses from drinking water, but they may still remove significant numbers of the microorganisms, thus offering the user a significant level of protection. Verifications following an EPA approved test/QA plan serve to notify the public of the possible level of protection against biological contamination agents afforded to them by the use of a verified system.

1.3.1 Bacteria and Virus Surrogates

The expert technical panel recommended that NSF and USEPA use the bacteria *Brevundimonas diminuta* (American Type Culture Collection (ATCC) strain 19146, formerly *Pseudomonas diminuta*), as the surrogate for bacterial agents. This surrogate was chosen based on its small size, as the smallest identified bacterium of concern can be as small as 0.2 μm in diameter. *B. diminuta* has a minimum diameter of 0.2 to 0.3 μm (see section 5.5.3 for discussion about the bacteria cell sizes measured in the cultures used for this verification). *B. diminuta* is the accepted bacteria of choice for testing filters and membranes designed to remove bacteria. It is used in the American Society of Testing Materials (ASTM) "Standard Test Method for Retention Characteristics of 0.2-μm Membrane Filters Used in Routine Filtration Procedures for the Evaluation of Microbiological Water Quality" (2001).

NSF also used a genetically engineered strain of *B. diminuta*. The NSF Microbiology Laboratory inserted into a culture of *B. diminuta* strain 19146 a gene conferring resistance to the antibiotic kanamycin. This allowed the Microbiology Laboratory to use a growth media amended with 50 μg/mL of kanamycin to prohibit heterotrophic plate count (HPC) bacteria in the treated water samples from growing along with the kanamycin resistant *B. diminuta*.

The virus surrogates were the bacteriophages MS2, and fr. The ATCC designation and host *E. coli* strain for each virus is given Table 1-1.

Table 1-1. Virus and Host ATCC Designations

Virus	ATCC Designation	Host Bacteria ATCC Strain
MS2	ATCC 15597-B1	E. coli ATCC 15597
fr	ATCC 15767-B1	E. coli ATCC 19853

The expert technical panel recommended these viruses based on their small sizes and isoelectric points. The isoelectric point is the pH at which the virus surface is neutrally charged. MS2 is 24 nm in diameter with an isoelectric point at pH 3.9, and fr is 19 nm in diameter with an isoelectric point at pH 8.9. With varying isoelectric points, the viruses have different surface charges, or different strengths of negative or positive charge, depending on the pH. In solutions above the isoelectric point, the virus is negatively charged. Below the isoelectric point, the virus is positively charged. Using different pH settings for the virus challenges allowed an evaluation of whether electrostatic forces enhance virus retention in mechanical filtration scenarios. The pH 6 and 9 settings were chosen because they just are beyond the upper and lower boundaries for allowable pH in the USEPA National Secondary Drinking Water Regulations. The pH 7.5 setting was chosen because it is the midpoint between the boundaries.

The bacteria reduction challenges were performed only at pH 7.5, because the expert panel believed that bacteria cell size and mass are too large for electrostatic interactions to play a significant role in retention by the RO membrane

1.4 Testing Participants and Responsibilities

The ETV testing of the Watts Premier WP-4V was a cooperative effort between the following participants:

NSF
Watts Premier, Inc.
USEPA

The following is a brief description of each of the ETV participants and their roles and responsibilities.

1.4.1 NSF International

NSF is a not-for-profit organization dedicated to public health and safety, and to protection of the environment. Founded in 1946 and located in Ann Arbor, Michigan, NSF has been instrumental in the development of consensus standards for the protection of public health and the environment. The USEPA partnered with NSF to verify the performance of drinking water treatment systems through the USEPA's ETV Program.

NSF performed all verification testing activities at its Ann Arbor location. NSF prepared the test/QA plan, performed all testing, managed, evaluated, interpreted, and reported on the data generated by the testing, and reported on the performance of the technology.

Contact Information:

NSF International
789 N. Dixboro Road
Ann Arbor, MI 48105
Phone: 734-769-8010
Fax: 734-769-0109
Contact: Bruce Bartley, Project Manager
Email: bartley@nsf.org

1.4.2 Watts Premier

The verified system is manufactured by Watts Premier, a division of Watts Water Technologies. Watts Premier manufactures industrial, food service, POE, and POU water treatment systems

The manufacturer was responsible for supplying the test units, and for providing logistical and technical support as needed.

Contact Information:

Watts Premier Incorporated
1725 West Williams Drive
Suite C-20
Phoenix, AZ 85027
Phone: 800-752-5582
Fax: 623-931-0191
Contact Person: Mr. Bob Maisner
Email: maisnerr@wattsind.com

1.4.3 U.S. Environmental Protection Agency

USEPA, through its Office of Research and Development, has financially supported and collaborated with NSF under Cooperative Agreement No. R-82833301. This verification effort was supported by the DWS Center operating under the ETV Program. This document has been peer-reviewed, reviewed by USEPA, and recommended for public release.

Chapter 2
Equipment Description

2.1 RO Membrane Operation

Membrane technologies are among the most versatile water treatment processes with regard to their ability to effectively remove a wide variety of contaminants. RO membranes operate by the principal of cross-flow filtration. In this process, the influent water flows over and parallel to the filter medium and exits the system as reject water. Under pressure, a portion of the water diffuses through the membrane becoming “permeate”. Membrane pore sizes are small enough to reject bacteria and viruses by size exclusion, but they may still pass through imperfections in the membrane, or go around the membrane due to microscopic seal leaks.

2.2 Equipment Capabilities

The WP-4V is certified by NSF to *NSF/ANSI Standard 58 – 2006, Reverse Osmosis Drinking Water Treatment Systems* (NSF 2006). It has a certified production rate of 9.06 gallons per day This measurement is based on system operation at 50 pounds per square inch (psi) inlet pressure, a water temperature of 25°C, and a total dissolved solids (TDS) level of 750 ± 40 mg/L. The amount and quality of treated water produced varies depending on the inlet pressure, water temperature, and level of TDS. These measurements were not subject to verification during this study.

2.3 System Components

The WP-4V is a four-stage treatment system. Incoming water first passes through a sediment filter to remove particulate matter, such as rust and silt, and then through a carbon filter to remove chlorine or other contaminants. The third stage of treatment is the reverse osmosis membrane, which removes a wide variety of inorganic and larger molecular weight organic contaminants, protozoan cysts such as cryptosporidium and Giardia, and also bacteria and viruses to some degree. The permeate water is sent to a 3-gallon maximum capacity storage tank. Upon leaving the storage tank, the water passes through a second carbon filter to remove organic chemicals and other taste and odor causing substances before dispensing through the faucet. A photograph of the system is shown in Figure 2-1, and a parts diagram is shown in Figure 2-2.

Please note that this description, and the system operation description in section 2.4 are given for informational purposes only. This information was not subject to verification.

2.4 Other Same or Similar Models

Watts Premier markets other models that are either identical to the WP-4V, except in name, or that are identical to the WP-4V except for different pre-membrane or post-membrane filters. The

WP-4V was tested without any pre-membrane or post-membrane filters in place (see Section 3.1 for further discussion), so the results of this verification also apply to the following models:

WP-5	RO-4M
KP-5	WP-4
RO-5M	WP-4BVC
KP-4	WP—BVC-5

NSF has verified that the RO membranes in these models are identical, and function identically, to the RO membrane in the WP-4V.

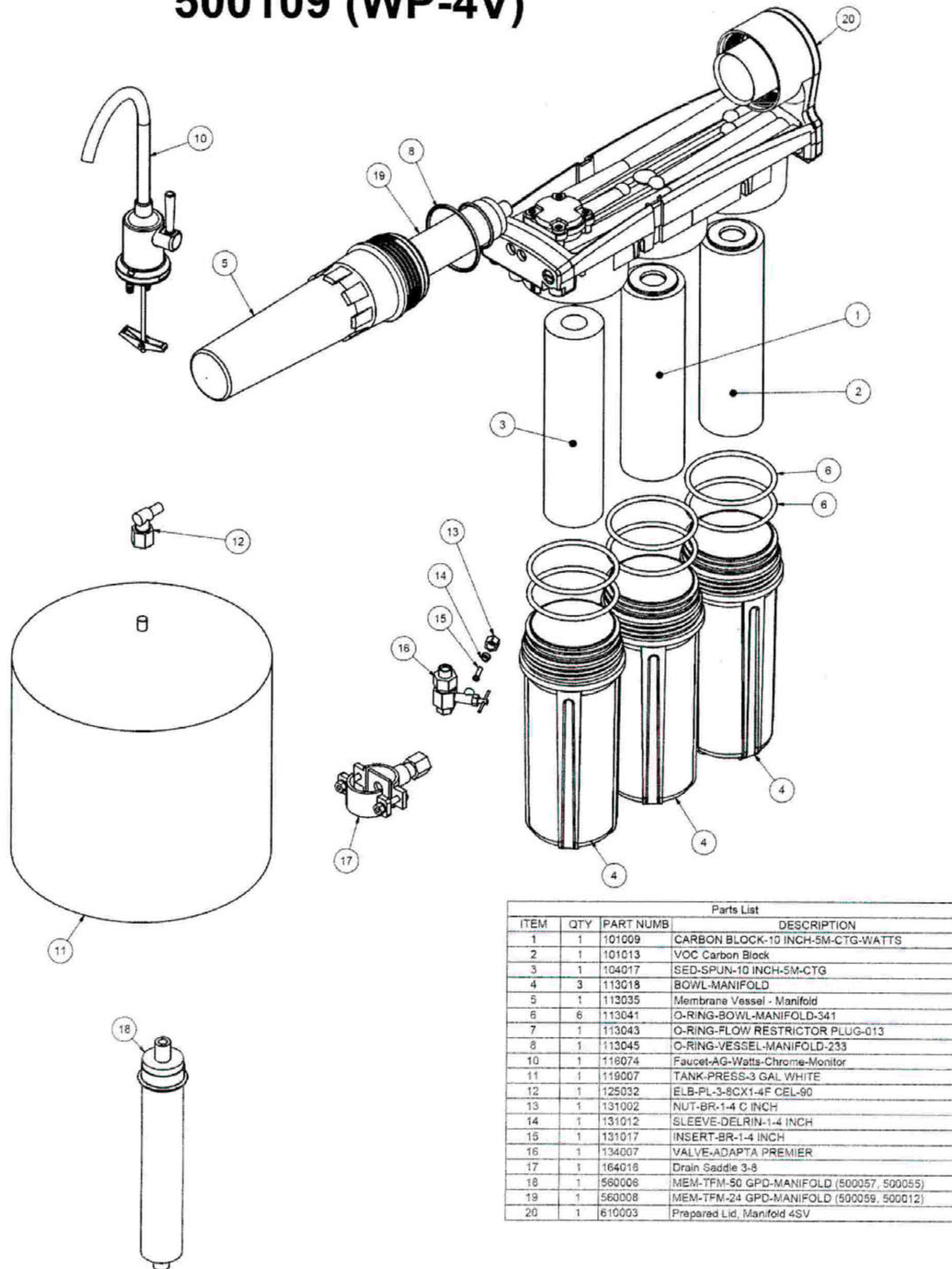
Figure 2-1. Photograph of the WP-4V System

2.5 System Operation

When the flow of water into the system is started, treated water will be continually produced until the storage tank is nearly full. At that time, the water pressure in the tank causes an automatic shut-off valve to stop the flow of water through the system. After a portion of the water is dispensed from the tank, the shut-off valve deactivates, allowing water to once again flow through the RO membrane into the storage tank.

The operational storage tank capacity will vary slightly from unit to unit, and may also be affected by the inlet water pressure. The storage tank capacity was measured to be 2.64 gallons when the system was tested for NSF/ANSI Standard 58 certification.

Figure 2-2. Parts Diagram of the WP-4V



Parts List			
ITEM	QTY	PART NUMB	DESCRIPTION
1	1	101009	CARBON BLOCK-10 INCH-5M-CTG-WATTS
2	1	101013	VOC Carbon Block
3	1	104017	SED-SPUN-10 INCH-5M-CTG
4	3	113018	BOWL-MANIFOLD
5	1	113035	Membrane Vessel - Manifold
6	6	113041	O-RING-BOWL-MANIFOLD-341
7	1	113043	O-RING-FLOW RESTRICTOR PLUG-013
8	1	113045	O-RING-VESSEL-MANIFOLD-233
10	1	116074	Faucet-AG-Watts-Chrome-Monitor
11	1	119007	TANK-PRESS-3 GAL WHITE
12	1	125032	ELB-PL-3-8CX1-4F CEL-90
13	1	131002	NUT-BR-1-4 C INCH
14	1	131012	SLEEVE-DELRIN-1-4 INCH
15	1	131017	INSERT-BR-1-4 INCH
16	1	134007	VALVE-ADAPTA PREMIER
17	1	164016	Drain Saddle 3-8
18	1	560006	MEM-TFM-50 GPD-MANIFOLD (500057, 500055)
19	1	560008	MEM-TFM-24 GPD-MANIFOLD (500059, 500012)
20	1	610003	Prepared Lid, Manifold 4SV

2.6 Rate of Waste Production

The rate of reject water production was measured during the certification process for NSF/ANSI Standard 58 certification. The efficiency rating, as defined by Standard 58 is the percentage measure of the amount of influent water delivered as permeate under a closed permeate discharge set of actual use conditions. The efficiency rating of the WP-4V is 8.4%, which means the system produces approximately 11 gallons of reject water for each gallon of product water produced. The efficiency rating was not verified as part of this evaluation.

2.7 Equipment Operation Limitations

Watts Premier gives the following limitations for the drinking water to be treated by the system:

- temperature of 40 – 100°F;
- pressure of 40 – 100 psig;
- pH of 3 – 11;
- maximum TDS level of 1,800 mg/L;
- maximum water hardness of 10 grains per gallon (1 grain per gallon equals 17.1 mg/L of hardness, expressed as calcium carbonate equivalent) may reduce membrane life; and
- no iron present.

2.8 Operation and Maintenance Requirements

The following are the operation and maintenance requirements specified in the product owner's manual:

- Replacement of the pre-membrane sediment and pre-membrane carbon filter every 12 months;
- Replacement of the RO membrane every 2 to 5 years (Watts Premier offers free treated water TDS analysis for monitoring membrane operation, or the user can purchase a TDS monitor);
- Replacement of the post-membrane carbon filter every 12 months or after 600 gallons have been treated;
- Annual sanitization of the system with hydrogen peroxide or bleach is recommended; and
- The flow restrictor plug must be cleaned each time the RO membrane is replaced.

The WP-4V system relies on the user to determine when the filters and RO membrane need to be replaced. There are no on-line monitors or indicators built into the system to track the volume of water treated. However, to compensate for this, for NSF/ANSI Standard 58 certification the post-membrane carbon filter was tested out to 200% of the claimed capacity, as opposed to 120% of capacity for systems with volume-based monitors.

Chapter 3
Methods and Procedures

3.1 Introduction

The challenge tests followed the procedures described in the *Test/QA Plan for Verification Testing of the Watts Premier WP-4V Point-of-Use Drinking Water Treatment System for Removal of Biological Contamination Agents*.

Five WP-4V systems were tested. As described in Section 2.3, the WP-4V employs an RO membrane, a sediment filter, and carbon filters to treat drinking water. However, the systems were tested with only the RO membrane in place. The sediment and carbon filters do not have pore sizes small enough to remove bacteria or viruses, but they could temporarily retain significant numbers of the organisms through electrostatic interactions, giving a positive bias to the performance data. Otherwise the systems were operated as sold to the consumer.

3.2 Verification Test Procedure

3.2.1 Test Rig

The five systems to be tested were plumbed to a single test station such that they were all attached to the same influent feed line. The test station used a 500-gallon polyethylene tank to hold the influent challenge water. See Figure 3-1 for a schematic diagram of the test rig. Figure 3-2 shows the systems installed on the test rig.

3.2.2 Test Rig Sanitization

The test apparatus was sanitized prior to the installation of the test systems to keep the heterotrophic bacteria population to a minimum. After sanitization, the test apparatus was flushed until a less-than-detectable concentration of sanitizing agent was present.

3.2.3 Test Water

3.2.3.1 Base Water

Ann Arbor, Michigan municipal drinking water was deionized to make the base water for the tests. The base water had the following constraints:

- Conductivity ≤ 2 μS/cm at 25°C;
- Total chlorine ≤ 0.05 mg/L;
- TOC < 100 μg/L; and
- Heterotrophic bacteria plate count < 100 colony forming units (CFU)/mL.

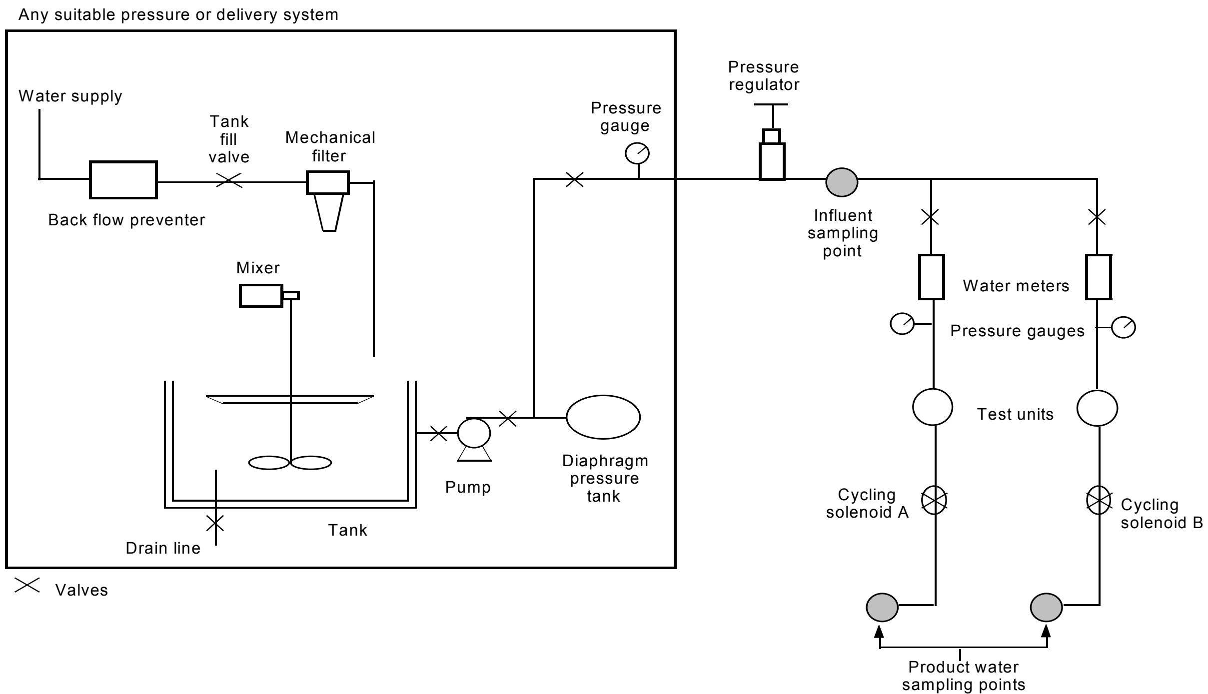
The base water was then adjusted to meet the following characteristics:

- Addition of sodium bicarbonate to achieve an alkalinity (expressed as calcium carbonate) of 100 ± 5 mg/L prior to pH adjustment;
- pH adjustment with hydrochloric acid or sodium hydroxide to reach a value of 6.0 ± 0.5, 7.5 ± 0.5, or 9.0 ± 0.5 as required by challenge protocol; and
- Temperature of 25 ± 2°C.

Sodium chloride was also added to the water to achieve a target level of 750 mg/L of TDS. Please note that the test/QA plan did not specify that any TDS be added to the base water, and it also specified a water temperature of 20°C instead of 25°C. The water specifications were changed so that the testing laboratory could use the NSF/ANSI Standard 58 TDS reduction test water, which was already being produced for other testing activities. These deviations are discussed further in Section 5.9 on page 21.

The test water was made fresh for each challenge in 200-gallon volumes. Each batch was analyzed for alkalinity, pH, temperature, total chlorine, total hardness, TDS, and turbidity.

Figure 3-1. Schematic Diagram of Test Rig



3.2.3.2 Bacteria and Virus Challenges

The viruses were purchased from Biological Consulting Services of North Florida, and the bacteria from ATCC. The viruses were purchased in adequate volumes so that volumes of the suspensions received were added directly to the base test water. The bacteria were cultivated at NSF to obtain the challenge suspensions. Section 3.3.2.3 describes the method used to create the bacteria challenges.

The targeted influent challenge concentrations were 1×10^5 CFU per 100 milliliters, or greater, for *B. diminuta*, and 1×10^4 plaque forming units (PFU) per milliliter, or greater, for the fr and MS2 viruses. See Appendix A for influent challenge data.

Figure 3-2. Systems Installed on Test Rig

Separate challenges were conducted for the "normal" *B. diminuta* and kanamycin resistant *B. diminuta*, but both viruses were mixed together into one challenge. After addition of the challenge organism(s) to the base test water, the resultant challenge water was mixed for a minimum of 30 minutes using a recirculation pump prior to beginning the test.

3.2.4 System Operation

3.2.4.1 System Installation

The test systems were installed on a test rig by a NSF laboratory technician following the instructions in the WP-4V owner's manual. After installation, the systems were conditioned according to the vendor's instructions (filling the storage tanks and dispensing the contents to drain three times) using the base test water in Section 3.2.3.1 at pH 7.5 ± 0.5. At the end of the conditioning procedure, treated water samples were collected from each system as negative controls and analyzed for the challenge organisms.

3.2.4.2 TDS Reduction System Check

After completion of the vendor's conditioning procedure, the membranes underwent a TDS reduction test using the test protocol in NSF/ANSI Standard 58, modified so that the systems were operated for only one tank-fill period. Influent water samples and treated water samples from each system were collected and analyzed for TDS. Each system had to remove at least 75% of the TDS (the pass/fail point for NSF/ANSI Standard 58 certification) to be used for testing. This test ensured that the products undergoing verification testing were representative of the expected performance of the system, and that there were no membrane integrity or membrane seal problems. All systems passed this test, see Section 4.1 for the test data.

3.2.4.3 Additional Conditioning

After the TDS reduction system check test was complete, the RO membranes were operated using the base test water in Section 3.2.3.1 at pH 7.5 ± 0.5 for 5 more days prior to challenge testing. On each day the systems were operated continuously at a dynamic inlet pressure of 60 ± 3 psig for one tank-fill period. The systems then sat idle overnight under pressure, and the tanks were emptied the next morning to resume system operation.

Previous POU RO system ETV tests indicated that perhaps membrane performance does not stabilize until after four or five days, or four or five tank fills, of conditioning. Five extra days of conditioning ensured that the membranes were performing optimally prior to the chemical challenges.

3.2.4.4 Challenge Testing

Following the conditioning period, the RO membranes were challenged according to the schedule in Table 3-1. The test plan called for both the normal *B. diminuta* and kanamycin resistant *B. diminuta* challenges to be conducted prior to beginning the virus challenges, but the kanamycin resistant *B. diminuta* challenge was delayed to give the Microbiology Lab more time to grow the challenge suspensions.

Table 3-1. Challenge Schedule

Day	Surrogate Challenge	pH
1	*B. diminuta*	7.5 ± 0.5
2	fr and MS2	6.0 ± 0.5
3	fr and MS2	7.5 ± 0.5
4	Kanamycin resistant *B. diminuta*	7.5 ± 0.5
5	fr and MS2	9.0 ± 0.5

At the end of the workday before each challenge, or the morning of the challenge, a tank of the test water without challenge organisms was prepared as described in section 3.2.3.1. Prior to beginning each challenge, the pH was checked and adjusted, if necessary, and the bacteria or viruses were added as described in section 3.2.3.2.

Influent samples were collected at the start and end of each challenge for bacteria or virus enumeration, and for water chemistry analysis. Each system was operated continuously for one tank-fill period (approximately 4 to 5 hours).

After all systems shut off, the storage tanks were emptied into separate sterile containers, and samples were collected in sterile polypropylene bottles for challenge organism enumeration. The sample volumes were 1 L for the bacteria challenges, and 150 mL for virus challenges. All samples for bacteria or virus enumeration were enumerated in triplicate.

Following each challenge, the systems were flushed for one tank-fill period using the base test water without the test organism(s) included. The systems rested under pressure overnight, and the morning of the next challenge the storage tanks were emptied into sterile containers, and negative control samples were collected for analysis of that day's challenge organism(s). The negative control samples for the first *B. diminuta* challenge were collected after the last day of the conditioning period.

3.3 Analytical Methods

3.3.1 Water Quality Analytical Methods

The following are the analytical methods used during verification testing. All analyses followed procedures detailed in NSF Standard Operating Procedures (SOPs).

- Alkalinity was measured according to EPA Method 310.2 with the SmartChem Discrete Analyzer. Alkalinity will be expressed as mg/L $CaCO_3$.
- pH measurements were made with a Beckman 350 pH meter. The meter was operated according to the manufacturer's instructions, which are based on Standard Method 4500-H^+.
- Water temperature was measured using an Omega model HH11 digital thermometer, or equivalent.

- TDS for the TDS reduction system check test was measured through conductivity according to Standard Method 2510 using a Fisher Scientific Traceable™ Conductivity Meter. This method has been validated for use with the test water; NSF uses this method for analysis of samples from TDS reduction tests under NSF/ANSI Standard 58.
- Total chlorine was measured according to Standard Method 4500-Cl G with a Hach Model DR/2010 spectrophotometer using AccuVac ampules.
- Total Hardness was measured according to USEPA method 310.1 using the SmartChem Discrete Analyzer.
- Turbidity was measured according to Standard Method 2130 using a Hach 2100N turbidimeter.

3.3.2 Microbiology Analytical Methods

3.3.2.1 Sample Processing, and Enumeration of Viruses

The viruses were enumerated using a double agar layer method published in *NSF/ANSI Standard 55 – 2004, Ultraviolet Microbiological Water Treatment Systems* for enumerating MS2. This method is similar to the double agar layer method in USEPA Method 1601.

Four to eighteen hours prior to sample processing, 100 µL of the appropriate host *E.coli* suspension was pipetted into tubes containing 10 mL of fresh Tryptic Soy Broth (TSB), and incubated at 35 °C. After incubation, 100 µL volumes of the resulting *E. coli* culture were transferred to sterile, capped test tubes.

All samples were enumerated in triplicate. All samples were serially diluted for enumeration, and the effluent samples were also enumerated directly. One milliliter volumes of the sample or dilution were pipetted into the *E. coli* suspension test tubes. The tubes were vortexed for a minimum of 30 seconds to "mate" the bacteria and virus, and then 4 mL of molten, tempered TSB plus 1% agar was added to each tube. These mixtures were then poured over Tryptic Soy Agar (TSA) plates, and allowed to solidify. The plates were incubated at 35°C for 18-24 hours. Virus plaques were counted using a Quebec Colony Counter.

3.3.2.2 Bacteria Cultivation

The bacteria were purchased from ATCC and rehydrated with nutrient broth. After 48 hours of incubation at 30°C, tubes containing 10 mL of TSB were inoculated with 100 µL of the nutrient broth suspension. These tubes were incubated for 48 hours at 30°C. After this incubation period, 100 µL of these suspensions were pipetted into new tubes containing 10 mL of fresh TSB. These tubes were then also incubated for 48 hours at 30°C. This process was repeated at least three times, up to a maximum of 30 times.

3.3.2.3 Preparation of *B. diminuta* Challenge Suspensions

To obtain the challenge suspensions, 1 mL of a 48-hour TSB culture was pipetted into an appropriate volume of Saline Lactose Broth (SLB). The SLB culture was incubated in a shaking

water bath at 30 °C for 48 hours. Cells were harvested after centrifugation at 3,000 revolutions per minute for 10 minutes. The supernatant was discarded and the pellet was resuspended in 100 mL of phosphate buffered dilution water (PBDW). The resulting challenge suspensions were refrigerated and added to the tank of test water within one hour. Samples of the challenge suspension were collected and enumerated according to the method in section 3.3.2.4.

The challenge preparation procedure was identical for both the normal *B. diminuta* and the kanamycin resistant *B. diminuta*, the only difference was that for the kanamycin resistant bacteria, the SLB was amended with 50 μg/L of kanamycin, and 10 μg/L of tetracycline.

3.3.2.4 Sample Processing and Enumeration of *B. diminuta*

All samples were enumerated in triplicate using a membrane filtration method based on Standard Method 9215 D. All samples were serially diluted for enumeration with sterile PBDW, and the effluent samples were also enumerated directly. For the influent samples, 1 mL volumes of either the straight sample or dilutions were pipetted into sterile glass vacuum filtration funnels, and 25 mL of PBDW was also poured into the funnels. For the effluent samples, 100 mL of the straight sample and the dilutions were pipetted into the funnels. The contents were then vacuum filtered through sterile 0.1 μm membrane filters. The funnels were rinsed three times with approximately 5 mL of PBDW, and the rinse water was also suctioned through the filters. The membrane filters were aseptically removed from the apparatuses and placed onto R2A agar plates. The plates were incubated at 30°C for 48 hours. Characteristic *B. diminuta* colonies were counted with a Quebec Colony Counter.

The sample processing and enumeration procedures were identical for both the normal *B. diminuta* and the kanamycin resistant *B. diminuta*, the only difference was that the R2A agar was amended with 50 μg/L of kanamycin, and 10 μg/L of tetracycline for enumeration of the kanamycin resistant bacteria.

Chapter 4
Results and Discussion

4.1 TDS Reduction

The performance data from the TDS reduction system check test described in 3.2.4.2 are presented in Table 4-1. The certified TDS reduction for the WP-4V is 97%. The five units did not meet that percent reduction, but they did all reduce the TDS of the challenge water by greater than 75%, thus meeting the requirement in the test/QA plan for use of each unit in the bacteria and virus challenges..

Table 4-1. TDS Reduction Test Results

	TDS (mg/L)	Percent Reduction
Influent	770	
Effluents:		
Unit 1	66	91
Unit 2	44	94
Unit 3	40	95
Unit 4	86	89
Unit 5	30	96

4.2 Bacteria Reduction

Presented in Table 4-2 are the $\log_{10}$ reduction data for the *B. diminuta* challenges. The influent and effluent triplicate bacteria counts are presented in Appendix A. The triplicate influent and effluent counts were averaged by calculating geometric means. The means were then $\log_{10}$ transformed and $\log_{10}$ reduction values were calculated for each test unit.

The challenge organisms were detected in the effluents for all units in both challenges except for Unit 2 in the “normal” *B. diminuta* challenge. Since the Unit 2 effluent count for kanamycin resistant *B. diminuta* was 4.3 logs, and all other effluent samples had bacteria counts greater than $1x10^4$, it is possible that there was a sampling or analysis error associated with the Unit 2 “normal” *B. diminuta* sample. Therefore, that sample was not included in the mean $\log_{10}$ reduction calculation for the *B. diminuta* challenge.

The minimum log reduction observed was 1.3, which equates to a 95% removal of the bacteria. The maximum observed log reduction, excluding the “non-detect” 6.4 $\log_{10}$ removal, was 3.1, equaling 99.9% removal. The geometric mean $\log_{10}$ reduction for both challenges combined was 1.9.

All negative control samples were non-detect for the bacteria.

Table 4-2. Bacteria Log Reduction Data

Challenge Organism	Log_{10} Influent Challenge	Geometric Mean Log_{10} Reductions Unit 1	Unit 2	Unit 3	Unit 4	Unit 5	Geometric Mean of the 5 Units
B. diminuta	6.4	1.8	6.4*	1.3	1.5	1.6	1.5
Kanmycin Resistant *B. diminuta*	7.2	1.4	2.9	2.6	2.6	3.1	2.4
						Overall Geometric Mean:	1.9

*Number not included in mean log reduction calculation.

4.3 Virus Reduction

The virus log_{10} reduction data are presented in Table 4-3. The influent and effluent triplicate PFU counts are presented in Appendix A. As was done for the bacteria, the triplicate influent and effluent counts were averaged by calculating geometric means. The means were then log_{10} transformed and log_{10} reduction values calculated for each test system.

The minimum observed log_{10} reduction was 1.2, and the maximum observed log_{10} reduction was 3.7. These log_{10} reductions correspond to percent reductions of 94% and 99.98%, respectively. The overall geometric mean log_{10} reductions were 2.5 for fr and 2.6 for MS2.

A visual comparison of the log_{10} reductions versus the challenge water pH shows the mean log_{10} reductions decreasing with increasing pH. However, an examination of the 95% confidence intervals around the means (see Appendix A for data) shows that the decreases are not statistically significant.

All negative control samples were non-detect for the viruses.

Table 4-3. Virus Log Reduction Data

Target pH	Initial Measured pH	Final Measured pH	Challenge Organisms	Log_{10} Influent Challenge	Geometric Mean Log_{10} Reduction Unit 1	Unit 2	Unit 3	Unit 4	Unit 5	Geometric Mean of the 5 Units
6.0 ± 0.5	6.1	6.5	fr	3.9*	1.8	3.1	3.6	3.4	3.0	2.9
			MS2	3.8*	2.3	3.4	3.7	3.6	2.9	3.1
7.5 ± 0.5	7.5	7.7	fr	4.5	1.9	2.4	2.3	3.1	2.8	2.5
			MS2	4.2	1.7	2.4	2.4	3.4	3.2	2.5
9.0 ± 0.5	8.9	9.0	fr	5.0	1.4	2.3	2.1	2.3	2.6	2.1
			MS2	4.6	1.2	2.4	2.0	2.3	3.0	2.1
								Overall Means:	fr	2.5
									MS2	2.6

* The mean influent challenge did not meet the 4 log_{10} requirement. The start-up influent samples were above $1x10^4$ PFU/mL requirement, but the end-of-challenge influent samples were not.

Chapter 5
QA/QC

5.1 Introduction

An important aspect of verification testing is the QA/QC procedures and requirements. Careful adherence to the procedures ensured that the data presented in this report was of sound quality, defensible, and representative of the equipment performance. The primary areas of evaluation were representativeness, precision, accuracy, and completeness.

Because the ETV was conducted at the NSF testing lab, all laboratory activities were conducted in accordance with the provisions of the *NSF International Laboratories Quality Assurance Manual* (NSF 2004).

5.2 Test Procedure QA/QC

NSF testing laboratory staff conducted the tests by following a USEPA-approved test/QA plan created specifically for this verification. NSF QA Department Staff performed an informal audit during testing to ensure the proper procedures were followed. The audit yielded no significant findings.

5.3 Sample Handling

All samples analyzed by the NSF Chemistry Laboratory were labeled with unique ID numbers. These ID numbers appear in the NSF laboratory reports for the tests. All samples were analyzed within allowable holding times.

5.4 Chemistry Analytical Methods QA/QC

The calibrations of all analytical instruments, and the analyses of all parameters complied with the QA/QC provisions of the *NSF International Laboratories Quality Assurance Manual*.

The NSF QA/QC requirements are all compliant with those given in the USEPA method or Standard Method for the parameter. Also, every analytical instrument has an NSF SOP governing its use.

5.5 Microbiology Laboratory QA/QC

5.5.1 Growth Media Positive Controls

All media were checked for sterility and positive growth response when prepared and when used for microorganism enumeration. The media was discarded if growth occurred on the sterility check media, or if there was an absence of growth in the positive response check. Both *E. coli* hosts for the viruses were plated on TSA and incubated with the virus enumeration plates during

sample enumeration as a second positive growth control. *B. diminuta* from the stock cultures was plated on R2A agar and incubated with the bacteria enumeration plates as a positive control.

5.5.2 Negative Controls

All samples were enumerated in triplicate. For each sample batch processed, an unused membrane filter and a blank with 100 mL of PBDW filtered through the membrane were also placed onto the appropriate media and incubated with the samples as negative controls. No growth was observed on any blanks.

5.5.3 Bacteria Cell Size

The theoretical minimum size for *B. diminuta* cells is 0.2 to 0.3 μm in diameter. Using the accepted method of growth in SLB media to obtain smaller cell sizes, the NSF Microbiology lab was able to achieve cells less than 0.5 μm in diameter. Samples from the stock cultures of both the normal *B. diminuta* and kanamycin resistant *B. diminuta* were examined microscopically with a Zeiss Axioskop 2 Plus, and measurements were taken using the accompanying Axiovision computer program. The measurements for each culture are presented below in Table 5-1.

Table 5-1. Bacteria Cell Size Measurements

	Normal *B. diminuta* Diameter (μm)	Kanamycin Resistant *B. diminuta* Diameter (μm)
	0.47	0.34
	0.38	0.34
	0.34	0.32
	0.44	0.30
	0.34	0.24
Average:	0.39	0.31
Standard Deviation:	0.06	0.04

5.6 Documentation

All laboratory activities were documented using specially prepared laboratory bench sheets and NSF laboratory reports. This documentation can be found in the appendices. Data from the bench sheets and laboratory reports were entered into Excel spreadsheets. These spreadsheets were used to calculate average influents and effluents, and $\log_{10}$ reductions for each challenge. One hundred percent of the data entered into the spreadsheets was checked by a reviewer to confirm all data and calculations were correct.

5.7 Data Review

NSF QA/QC staff reviewed the raw data records for compliance with QA/QC requirements. NSF ETV staff checked 100% of the data in the NSF laboratory reports against the lab bench sheets.

5.8 Data Quality Indicators

The quality of data generated for this ETV is established through four indicators of data quality: representativeness, accuracy, precision, and completeness.

5.8.1 Representativeness

Representativeness refers to the degree to which the data accurately and precisely represent the expected performance of the RO system under normal use conditions. The test protocol was designed to be a conservative evaluation of product performance. The test water was of very low turbidity to minimize the potential for microbial adhesion to suspended particles, which could enhance apparent log reduction. The surrogates were chosen because of their small size. The virus surrogate challenges were carried out at pH 6, 7.5, and 9 to assess whether pH affects the performance of the RO membrane.

Representativeness was ensured by consistent execution of the test protocol for each challenge, including timing of sample collection, sampling procedures, and sample preservation. Representativeness was also ensured by using each analytical method at its optimum capability to provide results that represent the most accurate and precise measurement it is capable of achieving.

5.8.2 Accuracy

Accuracy was quantified as the percent recovery of the parameter in a sample of known quantity. Accuracy was measured through use of both matrix spikes of a known quantity, and certified standards during calibration of an instrument. The following equation was used to calculate percent recovery:

$$\text{Percent Recovery} = 100 \times [(X_{known} - X_{measured})/X_{known}]$$

where: X_{known} = known concentration of the measured parameter
$X_{measured}$ = measured concentration of parameter

Accuracy of the benchtop chlorine, pH, TDS, and turbidity meters was checked daily during the calibration procedures using certified check standards. Alkalinity and total hardness were analyzed in batches. Certified QC standards and/or matrix spikes were run with each batch.

The percent recoveries of all matrix spikes and standards were within the allowable limits for all analytical methods.

5.8.3 Precision

Precision refers to the degree of mutual agreement among individual measurements and provides an estimate of random error. One sample per batch was analyzed in duplicate for the TDS measurements. Duplicate municipal drinking water samples were analyzed for pH, total

chlorine, and turbidity as part of the daily calibration process. One out of every ten samples for alkalinity and total hardness was analyzed in duplicate. Precision of duplicate analyses was measured by use of the following equation to calculate relative percent difference (RPD):

$$RPD = \left| \frac{S_1 - S_2}{S_1 + S_2} \right| \times 200$$

where:

S_1 = sample analysis result; and

S_2 = sample duplicate analysis result.

All RPDs were within NSF's established allowable limits for each parameter. Please note that samples from this evaluation for alkalinity, TDS, and total hardness were batched with other non-ETV samples. The duplicate analysis requirements apply to the whole batch, not just the samples from this ETV.

5.8.4 Completeness

Completeness is the proportion of valid, acceptable data generated using each method as compared to the requirements of the test/QA plan. The completeness objective for data generated during verification testing is based on the number of samples collected and analyzed for each parameter and/or method.

Table 5-2. Completeness Requirements

Number of Samples per Parameter and/or Method	Percent Completeness
0-10	80%
11-50	90%
> 50	95%

Completeness is defined as follows for all measurements:

$$\%C = (V/T) \times 100$$

where:

%C = percent completeness;

V = number of measurements judged valid; and

T = total number of measurements.

5.8.4.1 Completeness Measurements

- Five systems were tested, as called for in the test/QA plan, giving a completeness measurement of 100% for this category.

- All planned water chemistry samples were collected and analyzed, except that total chlorine was not analyzed on day three of the conditioning period. A total of 15 samples were to be collected for total chlorine over the course of the evaluation. The one missed sample gives a completeness of 93%, which is acceptable.
- All scheduled bacteria and virus samples were collected and analyzed with acceptable results.

5.9 Measurements Outside of the Test/QA Plan Specifications

As discussed in section 3.2.3.1, the test water used for this evaluation was the NSF/ANSI Standard 58 TDS reduction test water. This water differed from the water called for in the test/QA plan in that it had sodium chloride added for TDS, and the temperature was 25 °C instead of 20° C. These changes did not significantly affect the viability of the challenge organisms, since there was no significant decrease in organism concentrations from the first to the second influent samples (see Appendix A).

All other water chemistry measurements were within the allowable ranges.

The second influent samples for both viruses during the pH 6 virus challenge were below the minimum target level of $1x10^4$ PFU/mL (see Appendix A). However, the first influent samples were above the target level. The low second influent sample levels caused the overall mean influent to be below $1x10^4$ PFU/mL, but the low influents did not limit the log_{10} reduction numbers, since there were no effluents with virus counts <1 PFU/mL.

Chapter 6
References

American Society of Testing Materials (2001). D 3862-80, Standard Test Method for Retention Characteristics of 0.2-µm Membrane Filters Used in Routine Filtration Procedures for the Evaluation of Microbiological Water Quality.

NSF International (2004). *NSF International Laboratories Quality Assurance Manual.* Ann Arbor, NSF International.

NSF International (2004). *NSF/ANSI 55-2004, Ultraviolet microbiological water treatment systems*. Ann Arbor, NSF International.

NSF International (2006). *NSF/ANSI 58 – 2006, Reverse osmosis drinking water treatment systems*. Ann Arbor, NSF International.

USEPA (2004). Water Security Research and Technical Support Action Plan. EPA/600/R-04/063.

Appendix A
Bacteria and Virus Counts, and Water Chemistry Data

Bacteria Challenges Data

Brevundimonas diminuta				
Sample	Influent/Effluent Triplicate Counts (CFU/100mL)	Influent/Effluent Geometric Mean(CFU/100mL)	Log_{10} Influent/ Effluent	Log_{10} Reduction
First Influent	$2.9x10^6$, $2.3x10^6$, $1.9x10^6$	$2.3x10^6$		
Second Influent	$2.0x10^6$, $2.4x10^6$, $2.3x10^6$	$2.2x10^6$		
Influents Combined		$2.3x10^6$	6.4	
Effluents:				
Unit 1	$8x10^4$, $4x10^4$, $2x10^4$	$4x10^4$	4.6	1.8
Unit 2	<1, <1, <1	<1	0	6.4*
Unit 3	$7x10^4$, $1.0x10^5$, $1.9x10^5$	$1x10^5$	5.0	1.4
Unit 4	$6x10^4$, $8x10^4$, $1.0x10^5$	$8x10^4$	4.9	1.5
Unit 5	$9x10^4$, $5x10^4$, $6x10^4$	$6x10^4$	4.8	1.6
*Number not included in statistical calculations			Overall Mean	1.5

Kanamycin Resistant *Brevundimonas diminuta*				
Sample	Influent/Effluent Triplicate Counts (CFU/100mL)	Influent/Effluent Geometric Mean(CFU/100mL)	Log_{10} Influent/ Effluent	Log_{10} Reduction
First Influent	$1.70x10^7$, $1.63x10^7$, $1.63x10^7$	$1.65x10^7$		
Second Influent	$1.38x10^7$, $1.49x10^7$, $1.33x10^7$	$1.40x10^7$		
Influents Combined		$1.52x10^7$	7.2	
Effluents:				
Unit 1	$1.22x10^6$, $7.0x10^5$, $6.8x10^5$	$8.3x10^5$	5.9	1.3
Unit 2	$1.42x10^4$, $1.49x10^4$, $3.12x10^4$	$1.88x10^4$	4.3	2.9
Unit 3	$4.26x10^4$, $3.72x10^4$, $3.56x10^4$	$3.84x10^4$	4.6	2.6
Unit 4	$4.72x10^4$, $3.60x10^4$, $4.00x10^4$	$4.08x10^4$	4.6	2.6
Unit 5	$1.68x10^4$, $1.21x10^4$, $1.32x10^4$	$1.39x10^4$	4.1	3.1
			Overall Mean	2.4

Virus Challenges at pH 6

fr

Sample	Influent/Effluent Triplicate Counts (PFU/mL)	Influent/Effluent Geometric Mean (PFU/mL)	Log_{10} Influent/ Effluent	Log_{10} Reduction
First Influent	$1.13x10^4$, $1.21x10^4$, $1.16x10^4$	$1.17x10^4$		
Second Influent	$4.2x10^3$, $5.1x10^3$, $4.6x10^3$	$4.6x10^3$		
Influents Combined		$7.3x10^3$	3.9	
Effluents:				
Unit 1	98, 142, 111	117	2.1	1.8
Unit 2	6, 4, 9	6	0.8	3.1
Unit 3	1, 3, 5	2	0.3	3.6
Unit 4	5, 3, 2	3	0.5	3.4
Unit 5	12, 5, 6	7	0.9	3.0
			Overall Mean	2.9
			Standard Deviation	0.7
			95% Confidence Interval	1.7 – 4.1

MS2

Sample	Influent/Effluent Triplicate Counts (PFU/mL)	Influent/Effluent Geometric Mean (PFU/mL)	Log_{10} Influent/ Effluent	Log_{10} Reduction
First Influent	$1.18x10^4$, $1.02x10^4$, $1.08x10^4$	$1.09x10^4$		
Second Influent	$4.3x10^3$, $3.9x10^3$, $3.7x10^3$	$4.0x10^3$		
Influents Combined		$6.6x10^3$	3.8	
Effluents:				
Unit 1	29, 34, 39	34	1.5	2.3
Unit 2	3, 4, 2	3	0.5	3.4
Unit 3	1, 2, 1	1	0.1	3.7
Unit 4	4, 1, 1	2	0.2	3.6
Unit 5	10, 6, 8	8	0.9	2.9
			Overall Mean	3.1
			Standard Deviation	0.6
			95% Confidence Interval	2.1 – 4.2

Virus Challenges at pH 7.5

fr

Sample	Influent/Effluent Triplicate Counts (PFU/mL)	Influent/Effluent Geometric Mean (PFU/mL)	Log_{10} Influent/ Effluent	Log_{10} Reduction
First Influent	$3.2x10^4$, $7.1x10^4$, $2.9x10^4$	$4.0x10^4$		
Second Influent	$2.1x10^4$, $2.6x10^4$, $2.4x10^4$	$2.4x10^4$		
Influents Combined		$3.1x10^4$	4.5	
Effluents:				
Unit 1	302, 416, 512	401	2.6	1.9
Unit 2	131, 121, 142	131	2.1	2.4
Unit 3	112, 143, 190	145	2.2	2.3
Unit 4	17, 28, 35	26	1.4	3.1
Unit 5	54, 49, 38	47	1.7	2.8
			Overall Mean	2.5
			Standard Deviation	0.5
			95% Confidence Interval	1.7 – 3.3

MS2

Sample	Influent/Effluent Triplicate Counts (PFU/mL)	Influent/Effluent Geometric Mean (PFU/mL)	Log_{10} Influent/ Effluent	Log_{10} Reduction
First Influent	$1.21x10^4$, $1.37x10^4$, $1.61x10^4$	$1.39x10^4$		
Second Influent	$9.1x10^3$, $2.0x10^4$, $2.9x10^4$	$1.7x10^4$		
Influents Combined		$1.6x10^4$	4.2	
Effluents:				
Unit 1	322, 260, 310	296	2.5	1.7
Unit 2	67, 75, 60	67	1.8	2.4
Unit 3	45, 75, 78	64	1.8	2.4
Unit 4	6, 4, 12	7	0.8	3.4
Unit 5	5, 12, 16	10	1.0	3.2
			Overall Mean	2.5
			Standard Deviation	0.7
			95% Confidence Interval	1.3 – 3.7

Virus Challenges at pH 9

fr

Sample	Influent/Effluent Triplicate Counts (PFU/mL)	Influent/Effluent Geometric Mean (PFU/mL)	Log_{10} Influent/ Effluent	Log_{10} Reduction
First Influent	$1.39x10^5$, $1.05x10^5$, $1.12x10^5$	$1.18x10^5$		
Second Influent	$7.7x10^4$, $6.3x10^4$, $8.2x10^4$	$7.4x10^4$		
Influents Combined		$9.3x10^4$	5.0	
Effluents:				
Unit 1	$5.3x10^3$, $3.5x10^3$, $3.2x10^3$	$3.9x10^3$	3.6	1.4
Unit 2	443, 409, 600	477	2.7	2.3
Unit 3	990, 636, 572	711	2.9	2.1
Unit 4	672, 440, 492	526	2.7	2.3
Unit 5	290, 220, 216	240	2.4	2.6
			Mean	2.1
			Standard Deviation	0.5
			95% Confidence Interval	1.3 – 2.9

MS2

Sample	Influent/Effluent Triplicate Counts (PFU/mL)	Influent/Effluent Geometric Mean (PFU/mL)	Log_{10} Influent/ Effluent	Log_{10} Reduction
First Influent	$9.4x10^4$, $5.7x10^4$, $6.5x10^4$	$7.0x10^4$		
Second Influent	$2.9x10^4$, $1.5x10^4$, $3.7x10^4$	$2.5x10^4$		
Influents Combined		$4.2x10^4$	4.6	
Effluents:				
Unit 1	$2.9x10^3$, $2.1x10^3$, $2.8x10^3$	$2.6x10^3$	3.4	1.2
Unit 2	182, 116, 210	164	2.2	2.4
Unit 3	348, 500, 308	377	2.6	2.0
Unit 4	110, 220, 244	181	2.3	2.3
Unit 5	44, 28, 42	37	1.6	3.0
			Mean	2.1
			Standard Deviation	0.7
			95% Confidence Interval	0.9 – 3.3

RO Membrane Conditioning Water Chemistry Data

Sample	Day 1	Day 2	Day 3	Day 4	Day 5
Alkalinity (mg/L $CaCO_3$)	100	100	100	97	100
pH	8.0	7.5	7.7	7.9	7.5
Temperature (°C)	25	26	25	26	25
Total Chlorine (mg/L)	ND (0.05)	ND (0.05)	#	ND (0.05)	ND (0.05)
Total Hardness (mg/L $CaCO_3$)	6	6	8	6	ND (2)
TDS (mg/L)	870	830	670	780	880
Turbidity (NTU)	0.1	ND (0.1)	0.1	ND (0.1)	ND (0.1)

Sample not collected

RO Membrane Challenges Water Chemistry Data

Sample	B. diminuta	Kanamycin Resistant B. diminuta	pH 6 Viruses	pH 7.5 Viruses	pH 9 Viruses
Start-up Influent					
Alkalinity (mg/L $CaCO_3$)	100	100	61	97	120
pH	7.6	7.5	6.1	7.5	8.9
Temperature (°C)	25	26	25	25	26
Total Chlorine (mg/L)	ND (0.05)	ND (0.05)	ND (0.05)	ND (0.05)	ND (0.05)
Total Dissolved Solids (mg/L)	850	870	880	860	870
Total Hardness (mg/L $CaCO_3$)	8	ND (2)	ND (2)	ND (2)	ND (2)
Turbidity (NTU)	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)	ND (0.1)
2nd Influent					
pH	7.82	7.8	6.5	7.7	9.0
Temperature (°C)	25	24	24	24	25
Turbidity (NTU)	0.1	0.1	0.1	0.1	0.8