

Environmental Technology Verification Report

Inactivation of Microbiological
Contaminants in Drinking Water by
Ultraviolet Technology

NeoTech Aqua Solutions Inc.
Ultraviolet Water Treatment System
NeoTech D438™

Prepared by



NSF International

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

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FINAL

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Ultraviolet Light Technology**

NeoTech Aqua Solutions, Inc.

**Ultraviolet Water Treatment System
NeoTech D438™**

Prepared by:

**NSF International
Ann Arbor, Michigan 48105**

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

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Notice

The U.S. Environmental Protection Agency, through its Office of Research and Development, funded and managed, or partially funded and collaborated in, the research described herein. It has been subjected to the Agency's peer and administrative review and has been approved for publication. Any opinions expressed in this report are those of the author (s) and do not necessarily reflect the views of the Agency, therefore, no official endorsement should be inferred. Any mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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- Attachment 1 NeoTech Technical Manual and Documentation
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Abbreviations and Acronyms

A ₂₅₄	Absorbance at 254 nm
ATCC	American Type Culture Collection
°C	Degrees Celsius
CFU	Colony Forming Units
cm	Centimeter
DWS	Drinking Water Systems
EPA	U. S. Environmental Protection Agency
ETV	Environmental Technology Verification
°F	Degrees Fahrenheit
GP	Generic Protocol
gpm	Gallons per minute
h	Hours
HPC	Heterotrophic Plate Count
L	Liter
lbs	Pounds
LIMS	Laboratory Information Management System
Log I	Log inactivation
LT2ESWTR	Long Term 2 Enhanced Surface Water Treatment Rule
m	Meter
min	Minute
mJ	Millijoules
mg	Milligram
mL	Milliliter
MS2	MS2 coliphage ATCC 15597 B1
NeoTech	NeoTech Aqua Solutions, Inc. (formerly Ultraviolet Sciences, Inc.)
NIST	National Institute of Standards and Technology
nm	Nanometer
NRMRL	National Risk Management Research Laboratory
NSF	NSF International (formerly known as National Sanitation Foundation)
NTU	Nephelometric Turbidity Unit
ORD	Office of Research and Development
PFU	Plaque Forming Units
psi	Pounds per Square Inch
QA	Quality Assurance
QC	Quality Control
QA/QC	Quality Assurance/Quality Control
QAPP	Quality Assurance Project Plan
QMP	Quality Management Plan
RED	Reduction Equivalent Dose
RED _{meas}	Measured Reduction Equivalent Dose - from test runs
RED _{val}	Validated Reduction Equivalent Dose - based on selected pathogen and uncertainty
RPD	Relative Percent Deviation

SM	Standard Methods for the Examination of Water and Wastewater
SOP	Standard Operating Procedure
TQAP	Test /Quality Assurance Plan
TDS	Total Dissolved Solids
TSA	Tryptic Soy Agar
UVT	UV transmittance
TSB	Tryptic Soy Broth
µg	Microgram
µm	Microns
USEPA	U. S. Environmental Protection Agency
UVDGM	UV Design Guidance Manual 2006
U _{DR}	Uncertainty of collimated beam data
U _S	Uncertainty of sensor
U _{SP}	Uncertainty of set point
U _{VAL}	Uncertainty of validation

Chapter 1

Introduction

1.1 ETV Program Purpose and Operation

The U.S. Environmental Protection Agency (USEPA) has created the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved environmental technologies through performance verification testing 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 permittees; 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 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 Center (DWS) 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 the ETV testing was to validate using the set line approach the ultraviolet light (UV) dose delivered by the NeoTech Aqua Solutions Inc. (NeoTech) Ultraviolet Water Treatment System Model D438 (NeoTech D438™) as defined by these regulatory authorities and their guidelines and regulations:

- Water Supply Committee of the Great Lakes--Upper Mississippi River Board of State and Provincial Public Health and Environmental Managers otherwise known as The Ten States Standards 2012;
- The Norwegian Institute of Public Health (NIPH) and its guidelines; and
- The New York Department of Health (NYDOH) and its code.

Another purpose was to use the same data set to calculate the log inactivation of a target pathogen such as *Cryptosporidium* and *Giardia* using the *Generic Protocol for Development of Test / Quality Assurance Plans for Validation of Ultraviolet (UV) Reactors*, August 2011

11/01/EPADWCTR (GP-2011) which is based on *Ultraviolet Design Guidance Manual For the Long Term 2 Enhanced Surface Water Treatment Rule*, Office of Water, US Environmental Protection Agency, November 2006, EPA 815-R-06-007 (UVDGM-2006).

The setline approach was based on validation testing at three set points (a set point is defined as a single flow rate and irradiance output that delivers the targeted UV dose). The results of the three set point tests were used to develop a setline that defines the maximum flow rate - minimum irradiance output required to ensure the UV dose is achieved. The microorganism used for this validation test was MS2 coliphage virus (MS2). The target UV dose was a measured Reduction Equivalent Dose (RED_{meas}) of $>40 \text{ mJ/cm}^2$. This dose was calculated based on the understanding of dose calculations used internationally and by the Ten States Standards. The RED_{meas} was then adjusted based on the uncertainty of the measurements to calculate a MS2 based validated dose (RED_{val}) where the RED bias is set equal to one (1.0) in accordance with the unique approach of the State of New York. The RED_{meas} data were also adjusted for uncertainty and the *Cryptosporidium* and *Giardia* RED bias factors from UVDGM-2006 Appendix G. The data were used to estimate the log inactivation of *Cryptosporidium* and *Giardia* so that a regulatory agency could grant log credits under the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR). NeoTech selected flow rates of 150, 250, and 435 gpm as the target flow rates based on their design for NeoTech D438™.

Based on the results of the three set points, a setline was developed for this unit. During full-scale commercial operation, Federal regulations require that the UV intensity as measured by the UV sensor(s) must meet or exceed the validated intensity (irradiance) to ensure delivery of the required dose. Reactors must be operated within the validated operating conditions for maximum flow rate - minimum irradiance combinations, UV transmittance (UVT), and lamp status [40 CFR 141.720(d)(2)]. Under the UV setline approach, UVT does not have to be measured separately. The intensity readings by the sensor take into account changes in the UVT and the setline establishes the operating conditions over a range of flow rates used during the validation test.

This verification test did not evaluate cleaning of the lamps or quartz sleeves, nor any other maintenance and operation.

1.3 Verification Test Site

UV dose validation testing was performed at the NSF Testing Laboratory in Ann Arbor, Michigan. The NSF laboratory is used for all of the testing activities for NSF certification of drinking water treatment systems, and pool and spa treatment systems.

1.4 Testing Participants and Responsibilities

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

1.4.1 NSF International

NSF is an independent, not-for-profit organization dedicated to public health and safety, and to protection of the environment. Founded in 1944 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, MI location. NSF prepared the test/QA plan (TQAP), performed all testing, managed, evaluated, interpreted, and reported on the data generated by the testing, and reported on the performance of the technology.

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

1.4.2 U.S. Environmental Protection Agency

USEPA, through its Office of Research and Development (ORD), 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.

1.4.3 NeoTech Aqua Solutions, Inc. (formerly Ultraviolet Sciences, Inc.)

NeoTech supplied the UV test unit for testing, required reference sensors, detailed specifications on the equipment, UV lamps, lamp sleeves, and duty sensors, and written and verbal instructions for equipment operation. NeoTech also provided logistical and technical support, as needed.

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Email: info@neotechaqua.com
Website: neotechaqua.com

Chapter 2 Equipment Description

2.1 NeoTech General Information

NeoTech, headquartered in San Diego, California, designs and manufactures UV water treatment systems for disinfection, TOC reduction, and chlorine reduction purposes. NeoTech water treatment products are designed for industrial and commercial applications; pharmaceutical, microelectronics, beverage, pools/spas, hospitality, water reclamation, and small municipal drinking water delivery systems (<10 MGD).

The NeoTech team of scientists and engineers work in collaboration with a group of universities to develop more efficient UV systems for water purification applications. NeoTech has taken these innovations and applied them to a new product line of UV reactors.

NeoTech developed a highly reflective UV treatment chamber (US Patent 7,511,281) that maximizes the use of the UV light emitted by conventional mercury amalgam lamps. NeoTech has stated the following regarding the new treatment chamber:

The 99.8% reflective surface keeps the UV light inside the treatment chamber, reducing the amount of light energy necessary to achieve a proper UV dose. This reflective surface encapsulates the entire flow channel ensuring an even UV dose exists throughout the treatment chamber. No complicated internal baffling and mixing systems are necessary. This efficiency gain is unique to the NeoTech product line, resulting in smaller UV systems with fewer UV lamps to achieve a given UV dose. As a result NeoTech water treatment systems are more compact in design and require significantly less power to operate, saving the end user up to 90% in operating costs.

The NeoTech product line of treatment chambers was launched commercially in March 2009 after nearly two years of field trials.

2.2 NeoTech UV System Description

The NeoTech Ultraviolet Water Purification System validated in this test was the largest flow rated unit, NeoTech D438™. This unit is rated by NeoTech to handle 500 gpm. The system uses two low-pressure mercury amalgam lamps and one intensity sensor mounted in a stainless steel flow chamber. Figure 2-1 presents a picture of the system and basic dimensions of the system are shown in Figure 2-2. NeoTech provided an operating manual (Attachment 1 of this report), which included additional schematics and drawings with parts and dimensions of the reactor, the sensors, the lamps and the quartz sleeve placement. NeoTech has also provided additional information for the UV sensor (spectral data, measuring angle, measuring range, and output range) and for UV lamps (lamp life, irradiance output, power requirements, aging data, etc.) as required for the validation test. This information is presented in Attachment 2.

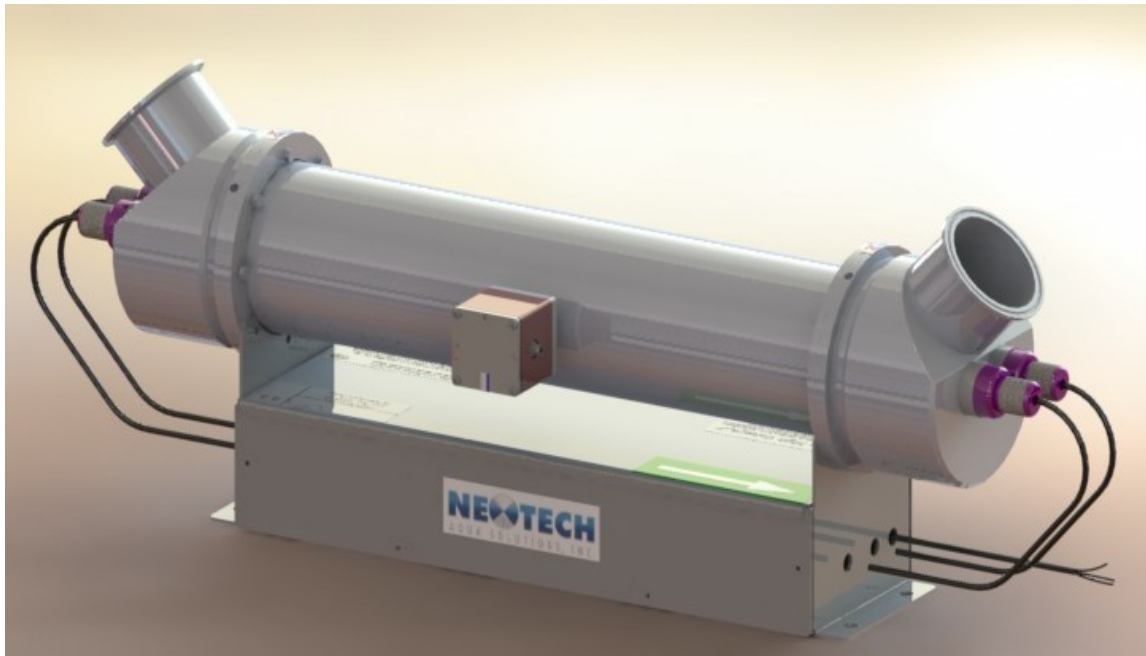


Figure 2-1. NeoTech D438™.

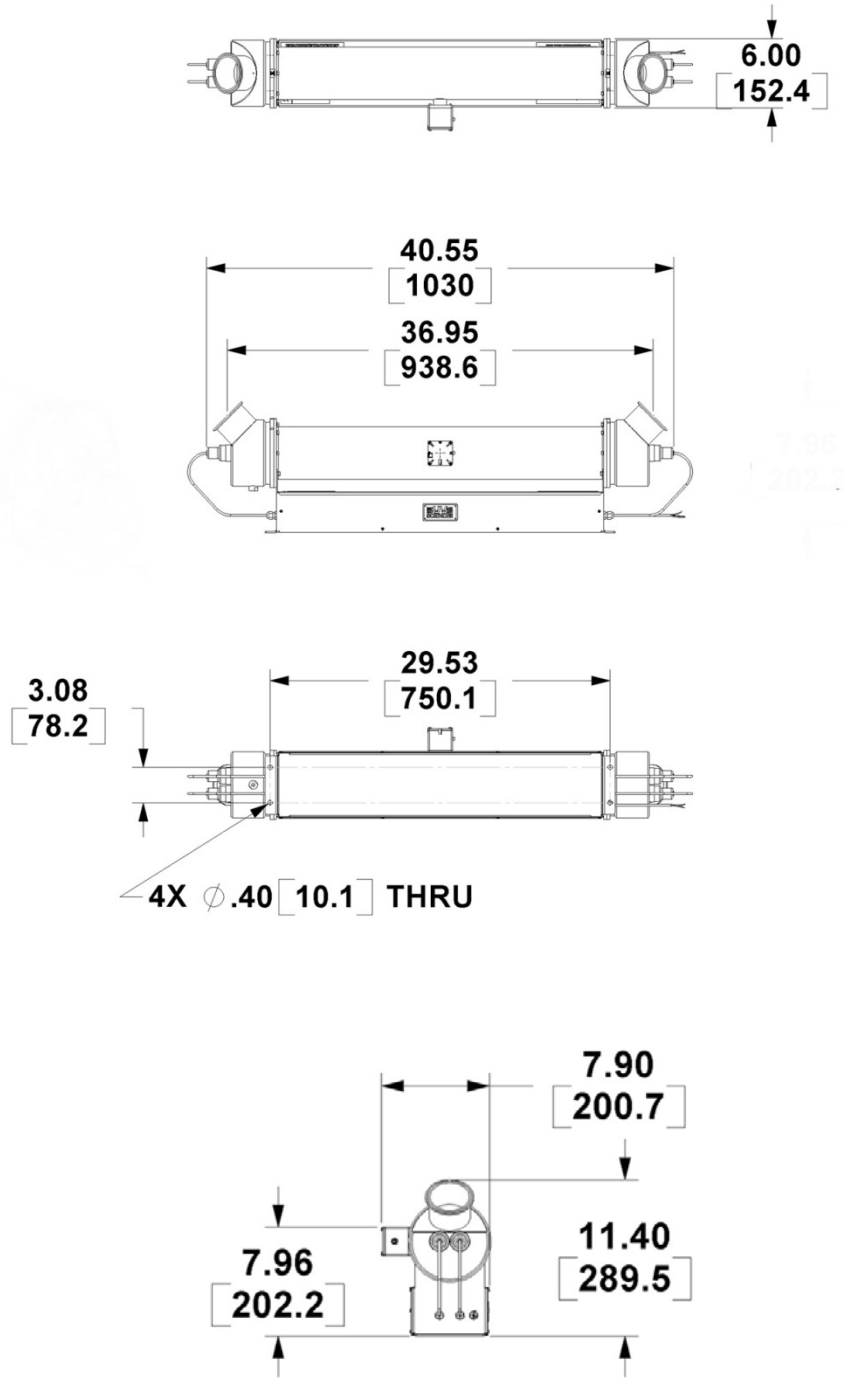


Figure 2-2. NeoTech D438™ Basic Dimensions.

2.3 NeoTech D438™ Specifications and Information

NeoTech provided the following information about their UV reactor:

Table 2.1. Basic UV Chamber Information

Manufacturer/Supplier	NeoTech Aqua Solutions, Inc.
Type or model	D438
Year of manufacture	2009
Maximum flow rate	500 gpm
Net dry weight	63 pounds
Volume	278.38 cu inches
Electrical power	120 VAC, 50/60Hz; 15 A max.
Operating power consumption	300 watts
Maximum pressure	150 psi static and transient
Ambient water temperature	35 °F (2 °C) min.; 80 °F (27 °C) max.
Maximum cleaning temperature	Steam sterilize with pure steam up to 257 °F for up to 90 minutes
Inlet pipe size	3 inches

Table 2.2. Low Pressure Lamp Information

Type	Low-pressure
Model	Light Sources, Inc. Model M1-4Y-01 NeoTech Part Number LK 38
Number of lamps per reactor	2
UV emission at wavelengths ranging from 240-290 nm	➤ 90% irradiance at 253-256 nm
Lamp life	9000 hrs – Aging data in Attachment 2
Power supply unit's name, make and serial numbers	Fulham WH5 or WH7 ballast 120 V; Power supply by NeoTech,
Irradiance @ 1m	334 (mW/cm ²)
UV output	35 W
Operating lamp watts	111 W
Lamp current and voltage	1.3 A; 86 V

Table 2.3. UV Lamp Sleeve Information

Type or model	Heraeus Quartz;
Quartz material	Supersil 310
Pressure resistance	1000 kPa

Table 2.4. UV Sensor Information

Type / model	UVIM-3 - 1660-002
Measuring field angle	180 degrees with cosine corrector cap, 64.7 degrees without cap
Number of sensors per reactor and placement	1
Signal output range	4 - 20 mA
Measuring range output signal	0 - 160 mW/cm ²

UV sensor spectral information has been provided by NeoTech to demonstrate the sensor meets the basic requirements of the *Generic Protocol for Development of Test/Quality Assurance Plans for Validation of Ultraviolet (UV) Reactors, 7/2010 (GP-2010)*. The GP-2010 and updated GP-2011 are based on the EPA's UVDGM-2006. These data are presented in Attachment 2. Sensor calibration information was provided by NeoTech prior to the start of the test. Sensor calibration was also checked with reference sensors as part of the test procedures. NeoTech has provided information on the calibration (NIST traceable) of the equipment used to calibrate the sensors. These data are provided Attachment 2.

2.4 NeoTech Ultraviolet Treatment System Standard Features

NeoTech has provided the following information on the features of the NeoTech series of reactors.

Standard Features of all NeoTech systems include:

- 316L and 304 stainless steel construction;
- Ra-15 finish for all wet contact surfaces;
- Low Pressure Amalgam lamps;
- Static Operation up to 1 hour (no flow);
- NIST traceable UV intensity monitor with LED output on control panel;
 - provides real-time lamp intensity information;
 - 4-20mA signal for data logging of UV intensity;
- High operating pressure (150 psig);
- NEMA 4X Control panel with remote monitoring and control capability;
 - 4-20mA scaled analog output;
 - Remote Shut off;
 - Alarms for lamp out and power off;
- Sanitary fittings and Viton gaskets;
- UV lamp replacement requires no tools required; and
- UL and CE approved. TUV verified.

Chapter 3 Methods and Procedures

3.1 Introduction

The tests followed the procedures described in the *Test/Quality Assurance Plan for The Ultraviolet Sciences Inc. Ultraviolet (UV) Water Purification System Model UVXS438S Reactor, August 2010* (TQAP). The TQAP was adapted from GP-2010. The GP-2010 and updated GP-2011 version are based on the EPA's UVDGM-2006. The TQAP is available from NSF upon request.

The approach used to validate UV reactors is based on biodosimetry, which determines the log inactivation of a challenge microorganism during full-scale reactor testing for specific operating conditions of flow rate, UVT, and UV intensity (measured by the duty sensor). A dose-response equation for the challenge microorganism (MS2 coliphage for this test) is determined using a collimated beam bench-scale test. The observed log-inactivation values from full-scale testing are input into the collimated beam derived-UV dose-response equations to estimate a RED. The RED value is adjusted for uncertainties and biases to produce the validated dose of the reactor for the specific operating conditions tested.

The methods and procedures were designed to accomplish the primary objective of the validation test of the NeoTech D438™, which was to develop a set line based on three set points (each set point is a specific flow rate- UV intensity combination) that would ensure a measured RED (RED_{meas}) of at least $40\text{mJ}/\text{cm}^2$ based on MS2 as defined by the "Ten States Standards". Test procedures were also designed so that the RED_{meas} could be adjusted based on the uncertainty of the measurements to calculate a MS2 based validated dose (RED_{val}) in accordance with the unique approach of the State of New York. The RED_{meas} data were also adjusted for uncertainty and the *Cryptosporidium* and *Giardia* RED bias factors from the UVDGM-2006 Appendix G. These validated RED data can be used by states to evaluate applicable log credits under the LT2ESWTR.

The GP-2010 requires the use of a second less sensitive challenge organism as part of the validation. T7 was initially included in the ETV UV Generic Protocol in the 2010 version as a result of research suggesting it could be a surrogate test microorganism with UV sensitivity similar to the UV sensitivity of *Cryptosporidium* (Fallon et.al, JAWWA, 99.3, March 2007). The GP-2010 technical advisory panel had reservations about using any test microorganism other than MS2 which has an excellent record of quality control response for collimated beam regression curves (Figure A.1 in the UVDGM-2006). The ETV GP-2010 technical advisory panel opinion was that other test microorganisms simply did not yet have the record of quality control limits as did MS2.

In 2010 during some initial validation studies, NSF attempted to use T7. The strain referenced by the JAWWA study (ATCC 11303-B7) was not available through ATCC. In fact, ATCC said verbally that the strain mentioned was not T7 and was not available. With the counsel of the EPA, NSF agreed to try using T7 ATCC BAA-1103-B38.

Comments in 2011 on the GP-2010 also found reasons not to specify only T7: “. However, T7 cannot be produced at nearly as high a titer as T1, so in the validation of high-flow reactors, replacing all the T1 test conditions with T7 test conditions would consume an unacceptable volume of raw phage stock.” Consequently the GP-2010 technical advisory panel recommended the use of any organism other than MS2 will be optional and the use of MS2 will be mandatory for all types of reactors. The use of a challenge organism other than MS2 will be determined by the consensus of stakeholders.

For the retesting done for this project, NSF chose to only use MS2 based on the concerns raised about T7 by reviewers and the changes made in the 2011 ETV UV Protocol (GP-2011). Instead, it was decided to illustrate how MS2 data was being used to satisfy many different regulatory requirements while using essentially the same data. The basic biodosimetry data was used to calculate the log inactivation of two target pathogens: *Cryptosporidium* and *Giardia*. The data was also used to calculate the 40mJ/cm² dose (RED_{meas}) requirement found in the "Ten States Standards" and the NIPH guidelines, and the “validated” dose approach (RED_{val}) used by the NYDOH.

UV reactor validation followed these steps:

1. Obtain the technical specifications for the system as provided by NeoTech;
2. Assessment of the UV sensors;
3. Collimated beam laboratory bench scale testing;
4. Full scale reactor testing;
5. Calculate the RED_{meas}; and
6. Adjust the RED_{meas} for uncertainty in UV dose and calculate a validated dose (RED_{val}) for *Cryptosporidium* and *Giardia* to show the log inactivation.

The target UV dosage was a RED_{meas} of ≥ 40 mJ/cm², based on MS2. NeoTech selected flow rates of 150, 250, and 435 gpm as the target flow rates based on their system design for NeoTech D438™ and screening and initial data from 2010.

3.2 UV Sensor Assessment

The NeoTech test unit duty sensor was evaluated according to the UV sensor requirements in the UVDGM-2006 prior to and following the verification testing. All UV intensity sensors (the duty and two reference sensors) were new sensors. Evidence of calibration of the sensors, traceable to NIST, was provided by NeoTech.

The validation testing requires confirmation of the duty sensor spectral response to assess whether the sensors are germicidal (see UVDGM-2006 Glossary for definition of germicidal) with a defined spectral response of at least 90% between 200 and 300 nm. The technical specifications of the NeoTech sensor and representation of sensitivity to the germicidal wavelength were provided by NeoTech and found to meet the requirements. The technical specifications of the NeoTech UV sensor and representation of sensitivity to the germicidal wavelength are included in Attachment 2.

During validation testing, the duty UV sensor measurement was compared to two reference sensor measurements to assure the duty sensor was within 10% of the average of the two reference sensor measurements.

The following steps were used to check the uncertainty of the duty and reference UV sensors. The sensors were checked before and after the validation testing.

1. Step 1: Water was passed through the reactor at the maximum UVT and the maximum lamp power setting to be used during validation testing.
2. Step 2: Using two calibrated reference UV sensors, each reference sensor was installed on the UV reactor at the sensor port. The UV intensity was measured and recorded.

Step 2 was repeated using the duty UV sensor.

3. Step 3: Steps 1 and 2 were repeated at maximum UVT and lamp power decreased to the minimum level expected to occur during validation testing.
4. Step 4: For a given lamp output and UVT value, the difference between the reference and duty UV sensor measurements were calculated as follows:

The absolute value of $[S_{\text{duty}} / S_{\text{Avg Ref}} - 1]$

Where:

S_{duty} = Intensity measured by a duty UV sensor,

$S_{\text{Avg Ref}}$ = Average UV intensity measured by all the reference UV sensors in the same UV sensor port with the same UV lamp at the same UV lamp power.

3.3 Headloss Determination

Headloss through the unit was determined over the range of expected flow rates, in this case from 150 gpm to 450 gpm. The inlet pressure near the inlet flange and the outlet pressure near the outlet flange were measured at several flow rates. Measurements were recorded for flow rates of 150, 250, 350 and 450 gpm. These data are reported Section 4.12.

3.4 Power Consumption Evaluation

The amperage and voltage used by the unit were measured during all reactor test runs.

Power data are presented in Section 4.11.

3.5 Feed Water Source and Test Rig Setup

The water source for this test was City of Ann Arbor, Michigan municipal drinking water. The water was de-chlorinated using activated carbon and confirmed by laboratory testing. For the lowered UVT conditions, the chemical Lignosulfonic Acid (LSA) was used to lower the UV transmittance to a level that achieved a duty sensor reading at the selected UV intensity set point.

LSA was added to the supply tank before each set of the lowered UVT runs and was well mixed using a recirculating pump system. UVT was measured continuously using an in-line UVT meter (calibrated daily) to confirm that proper UVT was attained.

NSF used a UV test rig and system setup that is designed to conform to the specifications described in Sections 5.4.3 and 5.4.4 of the UVDGM-2006. Figure 3-1 shows a basic schematic of the NSF test rig and equipment setup. The schematic is reproduced for informational purposes and is copyright protected. Figure 3-2 shows a photograph of the actual equipment and piping setup in the laboratory.

The feed water pump to the test unit was a variable speed pump. Flow rate was controlled by adjusting the power supplied to the pump and by a control valve. A magnetic water flow meter was used to monitor flow rate. The meter was calibrated and achieved the required accuracy of $\pm 5\%$. A chemical feed pump (injector pump) was used to inject MS2 coliphage upstream of an inline static mixer. The inline mixer ensured sufficient mixing of the microorganism prior to the influent sampling port, which was located upstream of the 90° elbow installed directly on the inlet to the unit. The effluent sampling port was located downstream of the test unit, downstream of a 90° elbow attached directly to the unit outlet and downstream of a second inline static mixer. This ensured good mixing of the treated water prior to the effluent sampling port. The 90° elbows prevented stray UV light from exiting the unit.

A power platform that measures amperage, volts, watts, and power factor was used to monitor power use by the test unit. The unit was wired into the platform and power consumption was recorded for each test run.

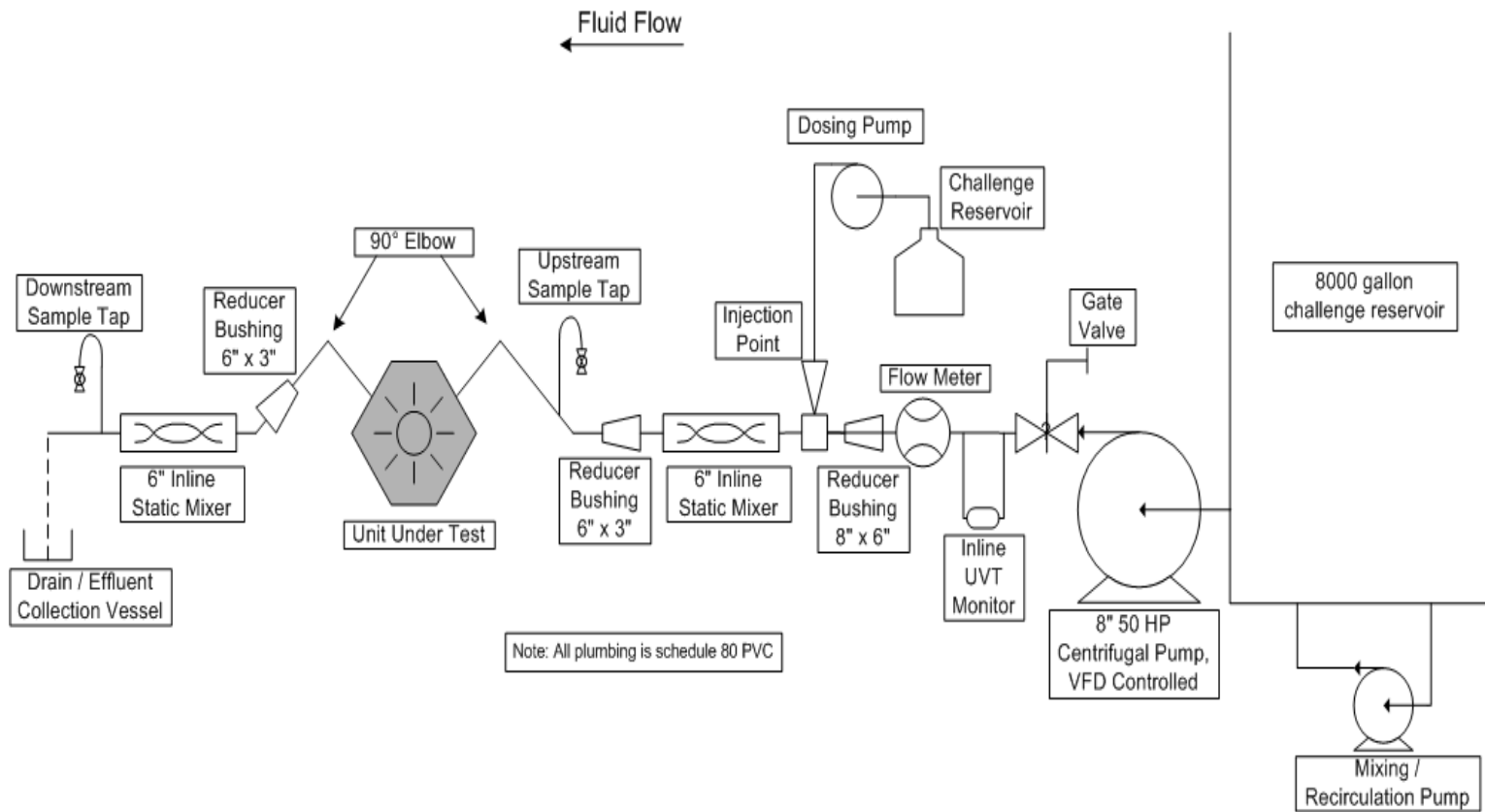


Figure 3-1. Schematic of NSF Test Rig[®].



Figure 3-2. Photograph of the Test Unit Setup.

3.6 Installation of Reactor and Lamp Burn-in

The UV reactor and the reactor inlet and outlet connections were installed at the NSF laboratory in accordance with the NeoTech installation and assembly instructions. Two 90° elbows, one upstream and one downstream of the unit, were used in the test rig setup to eliminate stray UV light. Figure 3-1 shows a schematic of the test rig setup and Figure 3-2 shows a photograph of the actual equipment setup. The UV lamps were new and therefore the system was operated for 100 hours prior to the start of the tests to provide proper burn-in of the lamps.

There was one duty sensor and two lamps in the NeoTech system. Therefore, the lamp positioning check requirements (i.e. checking each lamp and placing the lowest output lamp closest to the sensor) were required for this validation. The sensor readings were basically the same before and after the lamp positions were switched. The lamp positions that gave the highest sensor reading were used for all of the test runs. This provided the most conservative approach.

3.7 Collimated Beam Bench Scale Testing

The collimated beam procedure involves placing a sample collected from the test rig and containing MS2 in a petri dish, and then exposing the sample to collimated UV light for a predetermined amount of time. The UV dose is calculated using the measured intensity of the UV light, UV absorbance of the water, and exposure time. The measured concentration of microorganisms before and after exposure provides the “response,” or log inactivation of the microorganisms from exposure to UV light. Regression analysis of measured log inactivation for a range of UV doses produces the dose-response curve.

Appendix C of the UVDGM-2006 provides guidance on how to conduct the collimated beam bench-scale testing and produce a UV dose-response curve. The following sections describe the details of the collimated beam testing as performed by NSF.

3.7.1 Test Microorganism (Challenge)

MS2 coliphage (ATCC 15597-B1) was used in collimated beam bench scale testing and for the full-scale reactor dose validation tests.

MS2 coliphage ATCC 15597-B1 is a recommended microorganism for UV lamp validation tests. Further reasons for selecting this microorganism for UV validation are based on its inter-laboratory reproducibility (UVDGM-2006), ease of use and culturing, and demonstrated performance of MS2 in validation testing.

3.7.2 Test Conditions

The collimated beam tests were performed in duplicate at the minimum and maximum UVT test conditions. For this validation the testing spanned a period of two (2) days for the MS2 test runs, with Day 1 being the lowered UVT water tests and Day 2 the high UVT water lowered power tests. Collimated beam tests were run in duplicate on the minimum UVT water (90-91%) on Day 1. Collimated beam tests were run in duplicate on the maximum UVT water (97-98%) on Day 2. Thus, for this validation test, there are two sets of duplicate collimated beam test data for MS2, one at low UVT and one at high UVT.

For MS2, UV doses covered the range of the targeted RED dose, which in this case is 40mJ/cm². UV doses were set at 0, 20, 30, 40, 60 and 80 mJ/cm². The samples are clustered close to the 40mJ/cm² target dose with two doses above and below the target of 40mJ/cm².

The collimated beam radiometers were calibrated to ensure that the measured UV intensity met the criteria of an uncertainty of 8% or less at a 95% confidence level.

3.7.3 Test Apparatus

NSF uses a collimated beam apparatus that conforms to NSF/ANSI Standard 55[©] section 7.2.1.2. Attachment 3 includes a description of the apparatus and is reproduced for informational purposes and is copyright protected.

3.7.4 Collimated Beam Procedure

NSF collected two (2) one-liter samples from the influent sampling port of the test rig for collimated beam testing. Each bottle was used for one of the replicates for the collimated beam test. The MS2 spiked water was collected directly from the test rig each day during the test runs. The collimated beam test water and microorganism culture were the same as used in the full scale reactor tests.

NSF microbiological laboratory personnel followed the “Method for Challenge Microorganism Preparation, Culturing the Challenge Organism and Measuring its Concentration” in Annex A of NSF/ANSI Standard 55[©]. Please note that all reproduced portions of NSF/ANSI Standards are copyright protected.

For collimated beam testing of a water sample containing challenge microorganisms, NSF's laboratory followed this procedure:

1. Measure the A₂₅₄ of the sample.
2. Place a known volume from the water sample into a petri dish and add a stir bar. Measure the water depth in the petri dish.
3. Measure the UV intensity delivered by the collimated beam with no sample present using a calibrated radiometer using a calibrated UV sensor. The UV sensor is placed at the same distance from the radiometer as a sample.
4. Calculate the required exposure time to deliver the target UV dose described in the next section.
5. Block the light from the collimating tube using a shutter or equivalent.
6. Center the petri dish with the water sample under the collimating tube.
7. Remove the light block from the collimating tube and start the timer.
8. When the target exposure time has elapsed, block the light from the collimating tube.
9. Remove the petri dish and collect the sample for measurement of the challenge microorganism concentration. Analyze immediately or store in the dark at 4 °C (for up to 6 hours). Multiple dilutions are used to bracket the expected concentration range (e.g. sample dilutions of 10X, 100X, 1000X). Plate each dilution in triplicate and

calculate the average microbial value for the dilution from the three plate replicates that provide the best colony count.

10. Re-measure the UV intensity and calculate the average of this measurement and the measurement taken in Step 3. The value should be within 5% of the value measured in Step 3. If not, recalibrate radiometer and re-start at step 1.
11. Using the equation described in the next section, calculate the UV dose applied to the sample based on experimental conditions. The calculated experimental dose should be similar to the planned target dose.
12. Repeat steps 1 through 11 for each replicate and target UV dose value. Repeat all steps for each water test condition replicate.

The UV dose delivered to the sample is calculated using the following equation:

$$D_{CB} = E_s * P_f * (1-R) * [L * (1-10^{-A_{254} * d}) / (d + L) * A_{254} * d * \ln(10)] * t$$

Where:

D_{CB} = UV dose (mJ/cm²);

E_s = Average UV intensity (measured before and after irradiating the sample) (mW/cm²);

P_f = Petri Factor (unitless);

R = Reflectance at the air-water interface at 254 nm (unitless);

L = Distance from lamp centerline to suspension surface (cm);

d = Depth of the suspension (cm);

A_{254} = UV absorbance at 254 nm (unitless); and

t = Exposure time (s).

To control for error in the UV dose measurement, the uncertainties of the terms in the UV dose calculation meet the following criteria:

- Depth of suspension (d) \leq 10%
- Average incident irradiance (E_s) \leq 8%
- Petri Factor (P_f) \leq 5%
- $L/(d + L) \leq$ 1%
- Time (t) \leq 5 %
- $(1 - 10^{-ad})/ad \leq$ 5%

Further details and definitions of these factors are available in the collimated procedure and technical papers as referenced in the GP-2011 and UVDGM-2006. The QC data for these factors are presented in Section 5.5.3.

3.7.5 Developing the UV Dose- Response Curve

The collimated beam tests produced:

- UV Dose in units of mJ/cm²,

- Concentration of microorganisms in the petri dish prior to UV exposure (N_0) in units of plaque forming units (PFU)/mL, and
Concentration of microorganisms in the petri dish after UV exposure (N) in units of PFU/mL.

The procedure for developing the UV dose response curves was as follows:

1. For each UV test condition (high or low UVT water) and its replicate and for each day of testing, $\log N$ (PFU/mL) was plotted vs. UV dose (mJ/cm^2). A best fit regression line was determined and a common N_0 was identified as the intercept of the curve at UV dose = 0. A separate equation was developed for each UVT condition (lowest and highest). In this test, there were two days of testing for MS2, so there were two sets of data.
2. The log inactivation ($\log I$) was calculated for each measured value of N (including zero-dose) and the common N_0 identified in Step 1 using the following equation:

$$\log I = \log(N_0/N)$$

Where:

N_0 = The common N_0 identified in Step 1 (PFU/mL); and

N = Concentration of challenge microorganisms in the petri dish after exposure to UV light (PFU/mL).

3. The UV dose as a function of $\log I$ was plotted for each day of testing, and included water from both high and low UVT test conditions.
4. Using regression analysis, an equation was derived that best fit the data, forcing the fit through the origin. The force fit through the origin is used rather than the measured value of N_0 , because any experimental or analytical error in the measured value is carried to all the data points, adding an unrelated bias to each measurement. Using the y-intercept of the curve eliminates error carry through. The regression equation was then used to calculate the RED_{meas} for each full scale test sample.

The full set of collimated beam data and all calculations and regression analyses are presented in Chapter 4.

The regression analysis was used to derive an equation that best fits the data with a force fit through the origin. Both linear and a quadratic equations were evaluated to determine the best fit of the data. The regression coefficient, R^2 , was determined for each trend line and was considered acceptable if it was 0.9 or greater and for “r” \pm 0.95 or greater. The equation coefficients for each day were also evaluated statistically to determine which terms were statistically significant based on the P factor. A second order polynomial gave the best fit for the collimated beam dose response curves.

For this validation a single curve corresponding to one day’s worth of full scale reactor testing was used to calculate RED_{meas} values for that day. The higher UVT dose response curve was

used for the high UVT water day with reduced power and the lower UVT dose response curve was used for the day when the UVT of the test water was lowered with LSA.

3.7.6 Collimated Beam Data Uncertainty

The collimated beam data was fit to a polynomial regression and the uncertainty of the dose response equation based on a 95% confidence interval (U_{DR}) was calculated as follows:

$$U_{DR} = t * [SD / UV \text{ Dose}_{CB}] * 100\%$$

Where:

U_{DR} = Uncertainty of the UV dose-response fit at a 95% confidence level;

$UV \text{ Dose}_{CB}$ = UV dose calculated from the UV dose-response curve for the challenge microorganism;

SD = Standard deviation of the difference between the calculated UV dose response and the measured value, and

t = t-statistic at a 95% confidence level for a sample size equal to the number of test condition replicates used to define the dose-response.

The U_{DR} calculations and results are included in Section 4.4

3.8 Full Scale Testing to Validate UV Dose

3.8.1 Test Conditions for UV Intensity Set-Point Approach

The purpose of this testing was to determine a RED_{meas} dose of $\geq 40 \text{ mJ/cm}^2$ at three set points that were then used to establish a set line based on the three UV intensity and flow rate pairs. NeoTech specified the target flow rates (150, 250, 435 gpm) and UV target intensity levels (7.5, 10.0, 13.0 mW/cm^2) based on the results of screening test performed at NSF prior to the validation tests. The intensity targets were based on the expected intensity at UVTs of 91%, 94%, and 97%.

Each set point represents a given flow rate with testing under two conditions, (1) lowered UVT-maximum power and (2) high UVT-reduced power. The first test condition involved reducing the UVT until the UV intensity measured by the unit UV sensor equaled the target UV intensity set point. The second test condition was run with high UVT and with the power reduced until the unit UV intensity measured by the sensor was equal to the target UV intensity set point. Three target flow rates - intensity points (150 gpm - 7.5 mW/cm^2 ; 250 gpm - 10.0 mW/cm^2 ; 435 gpm - 13.0 mW/cm^2) were tested for the set line. All conditions were performed in duplicate. The intensity targets were based on expected intensity at UVT's of 91%, 94%, and 97%.

The LT2ESWTR requires validation of UV reactors to determine a log inactivation of *Cryptosporidium* or other target pathogens so that States may use the data to grant log credits. Therefore, in addition to determining the setline to achieve a minimum RED_{meas} of 40 mJ/cm^2 , additional calculations were run to show an example of determining the log inactivation of *Cryptosporidium* and *Giardia* by adjusting the RED_{meas} for uncertainty and RED bias.

A reactor control test (MS2 injection with the lamp off) was run at the low flow rate (150 gpm) and with high UVT water, which demonstrated that there was no inactivation of MS2 with the lamps off. A reactor blank was also run on each day of testing. The reactor blank was run with no phage injection at the low flow rate with the lamps at full power to demonstrate the testing system was low in MS2 concentration and other microorganisms. Reactor blank and control samples were collected in triplicate at the influent and effluent sampling locations and submitted for MS2 analyses.

Trip blanks were prepared and analyzed for each day of testing. The microbiology laboratory took two samples from the challenge solution prepared for one of the test runs. The first sample remained in the microbiology laboratory and the second sample traveled with challenge solution to the engineering laboratory and then was returned with the samples collected from the test run. Both samples were analyzed for MS2 and the results were compared to determine any change that might have occurred during transport of the samples. As with stability testing, trip blanks are important when samples must be shipped or carried long distance with the inherent holding time before delivery to the lab. At NSF, the test rig and laboratory are in the same building and the trip is "down the hall". Therefore, travel related impacts are of less concern, but trip blanks were run as part of the QC plan for these tests.

Table 3-1 shows a summary of the test conditions that were run for the validation test. A Sample and Analysis Management Program was also prepared and was provided to the NSF engineering and microbiology laboratories for use during the testing and for setting up the sample and analysis in the NSF sample management system.

Five sets of samples were collected at the influent and effluent sample ports for MS2 analysis during each test condition and it's duplicate. The delivered dose was calculated for each of the five samples and then the average of the five results was calculated to determine an average delivered dose (RED_{meas}).

Flow rate, intensity, and UVT data (from the NSF in-line UVT monitor) were collected at each of the five sample collection times for all test runs. These data were averaged to determine the average flow rate, UVT, and intensity for each test condition and it's duplicate.

In addition, samples for pH, turbidity, temperature, total and residual chlorine, *E coli*, and heterotrophic plate count (HPC) were collected at the influent and effluent sample ports once during each test run. Samples for iron (Fe) and manganese (Mn) analyses were collected once during each test run at the influent sample port to provide additional basic water quality data. Samples were also collected at the influent and effluent for UVT analysis by the chemistry laboratory bench scale spectrophotometer to confirm the in-line UVT measurements.

Table 3-1. Test Conditions for Validation

Validation Test	Flow Rate	UVT (%)	Lamp Power	Intensity Sensor Reading
Condition 1	150 gpm	91%	Maximum	Record actual reading
	250 gpm	94%		
	435 gpm	97%		
Condition 2	150 gpm	> 97%	Lowered to achieved intensity from Condition 1	Set to equal Condition 1 by lowering lamp power
	250 gpm	> 97%		
	435 gpm	>97%		
Condition 3 (reactor control)	150 gpm	>97%	Turned off	Not applicable
Condition 4 (reactor blank)	150 gpm	Daily Source water - ether high or low UVT	Full Power	Record

Condition 1 and 2 performed in duplicate
 Reactor blanks run for each day of testing
 UVT scan of feed water with and without UVT adjustment
 Trip blanks and method blanks run for each day of testing

3.8.2 Preparation of the Challenge Microorganisms

The challenge microorganism (MS2) used to validate the UV reactor was cultured and analyzed by NSF’s microbiology laboratory as specified in *Standard Methods for the Examination of Water and Wastewater*. NSF microbiological laboratory personnel followed the method for “Culture of challenge microorganism” in Annex A of NSF/ANSI Standard 55.

Propagation resulted in a highly concentrated stock solution (approximately 1.0×10^{12} PFU/mL) of essentially monodispersed phage whose UV dose-response follows second-order kinetics with minimal tailing. Over the range of RED values demonstrated during validation testing, the mean UV dose-response of the MS2 phage stock solution was within the 95-percent prediction interval of the mean response in Figure A.1 in Appendix A of the UVDGM-2006. Over a UV dose range of 0 to 120 mJ/cm^2 , the prediction intervals of the data shown in Appendix A of the UVDGM-2006 are represented by the following equations”

$$\text{Upper Bound: } \log I = -1.4 \times 10^{-4} \times UV \text{ Dose}^2 + 7.6 \times 10^{-2} \times UV \text{ Dose}$$

$$\text{Lower Bound: } \log I = -9.6 \times 10^{-5} \times UV \text{ Dose}^2 + 4.5 \times 10^{-2} \times UV \text{ Dose}$$

City of Ann Arbor tap water was filtered using activated carbon to remove any residual chlorine (confirmed by chemical analysis for total chlorine of the test water), organic surfactants and dissolved organic chemicals that may be UV absorbers. The filtered challenge water was then tested for the following parameters and found acceptable if the result is non-detectable or as otherwise indicated below:

- Total Chlorine;
- Free Chlorine;
- UV₂₅₄ ;
- UVT > 95%;
- Total Iron;
- Total Manganese;
- Turbidity ≤ 0.3 Nephelometric Turbidity Units (NTU);
- Total coliform (<1 CFU/100mL); and
- Heterotrophic Plate Count (<100 CFU/mL).

3.8.3 Testing – Measuring UV Dose

During full-scale reactor testing, the reactor was operated at each of the test conditions for flow rate, UVT, and lamp power as described in section 3.6.1. The following steps were taken to assure meeting data quality objectives:

1. Steady-state conditions were confirmed before injecting the challenge microorganism. Confirmation of steady state involved monitoring the flow rate, UV sensor measurements and the UVT to assure the test water and reactor meet the test conditions. After typically 3-5 minutes of operation and confirmation that UVT, sensor readings, and flow rate were steady, the injection pump was started and steady state conditions were demonstrated by waiting until the injection pump was at a steady flow rate based on measurements of weight loss of solution over 15-second time intervals. In all cases, sampling did not start until at least two minutes after the injection pump was started.
2. MS2 was injected into the feed water flow upstream of the reactor to achieve a concentration greater than 1×10^5 PFU/mL so that a minimum of a 4-log inactivation could be measured during the runs.
3. Sample taps remained open over the duration of the test.
4. Samples were collected in accordance with standards of good practice as defined by *Standard Methods* Section 9060.
5. Five (5) sample pairs were collected during approximately ten to twelve minutes of continuous flow at steady conditions. Each set of influent and effluent grab samples were collected as close in time as possible. The five sets of samples were spread out over the 10 to 12-minute continuous flow run.
6. Samples for assessing the challenge microorganism concentrations in the influent and effluent were collected in 125 mL bottles.
7. Samples were collected in bottles that have been cleaned and sterilized by the NSF laboratory.
8. Collected samples were delivered directly to the microbiological lab located in the same building after each sampling period. Sample analysis was generally started immediately, but could be stored in the dark and analysis started a few of hours later. All MS2 analysis was started within 4-6 hours of the time the samples were collected.

The following measurements and recordings were taken during each test run:

1. The flow rate through the reactor, UV sensor reading and on-line UVT measurements were recorded when each sample was collected during each run, yielding a minimum of five measurements for each test run.
2. Water chemistry and other microbiological grab samples were collected once per test condition after one of the challenge organism samples were collected. Samples for temperature, pH, *E. coli*, and HPC were collected at the influent and effluent locations, and samples for iron, manganese, turbidity and residual chlorine were collected at the influent location.
3. A sample for UVT was collected and measured by a UV spectrophotometer for each influent sample and at least one effluent sample;
4. A sample of the influent and effluent water was collected at the beginning of each test day and a UVT scan performed over the range of 200 to 400 nm, and
5. The electrical power consumed by system was recorded.

Chapter 4 describes the calculations and presents the data for determining the RED_{meas} and the validated dose (RED_{val}) at a each set point.

3.9 Analytical Methods

All laboratory analytical methods for water quality parameters are listed in Table 3-2.

Table 3-2. Analytical Methods for Laboratory Analyses

Parameter	Method	NSF Reporting Limit	Lab Accuracy (% Recovery)	Lab Precision (%RPD ⁽¹⁾)	Hold Time	Sample Container	Sample Preservation
Temperature	SM ⁽²⁾ 2550	-	-	-	-	-	-
pH	SM 4500-H ⁺ B		±1 SU of buffer	±0.1 SU	(3)	NA	None
<i>E. coli</i> / Total Coliform	SM 9223	1 CFU /100mL	-	-	24 hours	500 mL plastic	1% Tween 80
Iron	EPA 200.7	20 µg/L	70-130	10%	180 days	125 mL polyethylene	Nitric acid
Manganese	EPA 200.8	1 µg/L	70-130	10%	180 days	125 mL polyethylene	Nitric acid
Turbidity	SM 2130	0.1 NTU	95-105	-	(3)	NA	None
MS2	Top Agar Overlay	1 PFU/mL	-	-	24 hours	125 mL plastic	1% Tween 80
Absorbance UV ₂₅₄	SM 5910B	NA	60-140	≤ 20	2 days	1 L plastic	None
Residual chlorine	SM 4500-Cl D	0.05 mg/L	90-110	≤10%	(3)	NA	None
HPC	SM 9215B	1 CFU/mL	-	-	24 hours	125 mL plastic	1% Tween 80

(1) RPD = Relative Percent Deviation

(2) SM = Standard Methods

(3) Immediate analysis required

3.9.1 Sample Processing, and Enumeration of MS2:

MS2 sample processing and enumeration followed the procedures used in NSF/ANSI Standard 55.

3.9.2 Percent UVT Measurements:

The percent UVT for laboratory measurements was calculated from A_{254} . The equation for UVT using A_{254} is:

$$\text{UVT (\%)} = 100 * 10^{-A_{254}}$$

The on-line UVT analyzer provided immediate data throughout all test runs. The on-line analyzer was calibrated every day of operation. A primary standard was used at a minimum on the first day of testing. Daily calibration was performed on all test days using a certified secondary standard. Before the start of each day's testing a sample was taken to the laboratory and analyzed for direct comparison with the on-line analyzer to ensure the data were comparable.

All UVT measurements used a 1-cm path length and are reported on a 1-cm path length basis.

Spectrophotometer measurements of A_{254} were verified using NIST-traceable potassium dichromate UV absorbance standards and holmium oxide UV wavelength standards. The UV spectrophotometer internal QA/QC procedures outlined in the UVDGM-2006 were used to verify calibration. UV absorbance of solutions used to zero the spectrophotometer were verified using reagent grade organic-free water certified by the supplier to have zero UV absorbance.

The measurement uncertainty of the spectrophotometer must be 10% or less. To achieve this goal, the following procedures were used:

1. Verify that the spectrophotometer reads the wavelength to within the accuracy of a holmium oxide standard (typically ± 0.2 nm at a 95-percent confidence level),
2. Verify that the spectrophotometer reads A_{254} within the accuracy of a dichromate standard (e.g., 0.281 ± 0.005 at 257 nm with a 20 mg/L standard), and
3. Verify that the water used to zero the instrument has an A_{254} value that is within 0.002 cm^{-1} of a certified zero absorbance solution.

3.9.3 Analytical QA/QC Procedures

Accuracy and precision of sample analyses were ensured through the following measures:

- pH – Three-point calibration (4, 7, and 10) of the pH meter was conducted daily using traceable buffers. The accuracy of the calibration was checked daily with a pH 8.00 buffer. The pH readings for the buffer were within 10% of its true value. The precision of the meter was checked daily using duplicate synthetic drinking water samples. The difference of the duplicate samples was within ± 0.1 SU.

- Temperature – The thermometer used to give the reportable data had a scale marked for every 0.1°C. The thermometer is calibrated yearly using a Hart Scientific Dry Well Calibrator Model 9105.
- Total chlorine – The calibration of the chlorine meter was checked daily using a DI water sample (blank), and three QC standards. The measured QC standard values were within 10% of their true values. The precision of the meter was checked daily by duplicate analysis of synthetic drinking water samples. The RPD of the duplicate samples was less than 10%.
- Turbidity – The turbidimeter was calibrated as needed according to the manufacturer’s instructions with formazin standards. Accuracy was checked daily with a secondary Gelex standard. The calibration check provided readings within 5% of the true value. The precision of the meter was checked daily by duplicate analysis of synthetic drinking water samples. The RPD of the duplicate samples was less than 10% or had a difference of less than or equal to 0.1 NTU at low turbidity levels.

3.9.4 Sample Handling

All samples were labeled with unique identification numbers. These identification numbers were entered into the NSF Laboratory Information Management System (LIMS), and were used on the NSF lab reports for the tests. All challenge organism samples were stored in the dark at 4 ± 2 °C and processed for analysis within 4-6 hours.

3.10 Full Scale Test QA Controls

The following quality-control samples and tests for full-scale reactor testing were performed:

- *Reactor controls* – Influent and effluent water samples were collected with the reactor UV lamps in the reactor turned off. The change in log concentration from influent to effluent should correspond to no more than 0.2 log₁₀.
- *Reactor blanks* – Influent and effluent water samples were collected with no addition of challenge microorganisms to the flow passing through the reactor. Blanks were collected once on each day of testing. The reactor blank is acceptable when the MS2 concentration is less than 0.2 log₁₀.
- *Trip controls* – Trip controls were collected to monitor any change in challenge microorganisms during transport to the laboratory (in the same building).
- *Method blanks* – A sample bottle of sterilized reagent grade water was analyzed following the challenge microorganism assay procedure. The concentration of challenge microorganism with the method blank was non-detectable.
- *Stability samples* – Influent and effluent samples at low and high UVT were collected prior to the introduction of MS2. These samples were used to assess the stability of the challenge microorganism concentration and its UV dose-response over the time period from sample collection to completion of challenge microorganism assay. The challenge microorganism was added to achieve a concentration of approximately 1×10^3 PFU/L in the samples containing test water at the lowest and highest UVT. A sample was analyzed immediately (called time 0) and then 4 h, 8 h and 24 h after time 0. All analyses were performed in triplicate. While stability samples were performed during the test, they are

not directly applicable in this case as all sample analyses for MS2 were started within a 4 to 6 hours of collection.

3.11 Power Measurements

The voltmeter and ammeter used to measure UV equipment voltage and amperage had traceable evidence of being in calibration (e.g., have a tag showing that it was calibrated). Calibrations are performed at least yearly and all power equipment was calibrated within the past year.

3.12 Flow Rate

During validation testing, the QC goal was that the accuracy of flow rate measurements should be within $\pm 5\%$ of the true value. Flow meter accuracy was verified by monitoring the draw down volume in the supply tanks over time. The supply tanks have been calibrated using the catch and weigh technique. The flow meter was within 1.66% of the true value. Flow meter calibration data are presented in Section 5.6.

3.13 Evaluation, Documentation and Installation of Reactor

NeoTech provided technical information on the NeoTech D438™ and basic information on the UV lamps, sensor, and related equipment. An operating manual was provided. Additional information on the lamp output (confirmation of spectral output) was provided prior to the start of the validation test. All documentation and equipment data was reviewed prior to the start of testing. The following documentation was reviewed and found to conform to the GP-2011 and UVDGM-2006 requirements:

Reactor Specifications:

- Technical description of the reactor's UV dose-monitoring strategy, including the use of sensors, signal processing, and calculations (if applicable);
- Dimensions and placement of all wetted components (e.g., lamps, sleeves, UV sensors, baffles, and cleaning mechanisms) within the UV reactor;
- A technical description of lamp placement within the sleeve; and
- Specifications for the UV sensor port indicating all dimensions and tolerances that impact the positioning of the sensor relative to the lamps.

Lamp specifications:

- Technical description;
- Lamp manufacturer and product number;
- Electrical power rating;
- Electrode-to-electrode length; and
- Spectral output of the lamps (specified for 5 nm intervals or less over a wavelength range that includes the germicidal range of 250 – 280 nm and the response range of the UV sensors).

Lamp sleeve specifications:

- Technical description including sleeve dimensions;
- Materials of construction; and
- UVT at 254 nm for the medium pressure (MP) lamp with germicidal sensors.

Specifications for the reference and the duty UV sensors:

- Manufacturer and product number; and
- Technical description including external dimensions.

Sensor measurement properties:

- Working range;
- Spectral and angular response;
- Linearity;
- Calibration factor;
- Temperature stability; and
- Long-term stability.

Installation and operation documentation:

- Flow rate and pressure rating of the reactor;
- Assembly and installation instructions;
- Electrical requirements, including required line frequency, voltage, amperage, and power; and
- Operation and maintenance manual including cleaning procedures, required spare parts, and safety requirements.

Chapter 4

Results and Discussion

4.1 Introduction

The validation tests to demonstrate a minimum RED_{meas} of 40 mJ/cm^2 were run on August 2 and 3, 2012. The first day of testing was dedicated to the test conditions and duplicate runs where the UVT of the feed water was lowered to the target levels (91%, 94%, and 97%) and the lamps were operated at full power. The second day of testing was dedicated to the test conditions and duplicates where highest UVT feed water (97% target) was used and the lamp power was reduced to achieve the target intensity level. The test conditions and detail on the test rig setup, sampling procedures, and unit operation have been described in Chapter 3 Methods and Procedures.

All tests were conducted at the NSF laboratory in Ann Arbor MI and all analyses were performed by the NSF microbiological and chemistry laboratories at this location.

4.2 Sensor Assessment

The same duty sensor was used for monitoring intensity (irradiance) for all test runs. This sensor measured the intensity from the two low pressure lamps in the unit. The control panel provided direct readings of intensity in mW/cm^2 . This direct reading was based on converting the 4-20 mA output signal to intensity based on the calibration set by NeoTech.

The duty sensor was compared against two reference sensors to demonstrate that the duty sensor was within 10% of the average of the two reference sensors. This evaluation was conducted before and after the validation test runs, using the procedure described in Section 3.2. Table 4-1 presents the results of the sensor assessment. These data demonstrate that the duty sensor was within 10% of the average of the two reference sensors. The two reference sensors showed a variance of 2.9% at 100% power and 1.3% and 6.8% at reduced power.

Table 4.1. Sensor Assessment Data

Sensor	Intensity at 100% Power Before testing 90.5% UVT (mW/cm²)	Intensity at 100% Power After testing 98.2% UVT (mW/cm²)	Intensity with Power Reduced Before testing 90.5% UVT (mW/cm²)	Intensity with Power Reduced After testing 98.2% UVT (mW/cm²)
Reference #1 2012-08	8.6	17.0	3.4	7.8
Reference #2 2012-09	9.1	18.0	3.9	8.0
Average of Reference Sensor	8.85	17.5	3.65	7.9
Duty Sensor 2012-10	8.2	18.4	3.9	8.6
Deviation of Duty Sensor from Reference average	7.3	5.1	6.8	8.8

4.3 Collimated Beam Dose Response Data

Collimated Beam dose response data were generated for each of the test days for MS2 in accordance with the procedures described in Section 3.5. On test Day 1, collimated beam tests for MS2 were run on the minimum UVT water (91%). On test Day 2, collimated beam tests were run on maximum UVT water (97%). All collimated beam tests were performed in duplicate.

UV doses for the MS2 tests covered the range of the targeted RED dose, which in this case was 40mJ/cm². UV doses were set at 0, 20, 30, 40, 60 and 80 mJ/cm².

The collimated beam samples were collected directly from the test rig during the normal testing runs. Two 1 L bottles of the seeded influent water (MS2 was injected into the influent water during a test run) were collected to provide the two samples for duplicate analyses. Using this approach, the dose response data reflect the identical conditions to the biosimetric flow tests for sample matrix, UVT, and MS2 concentration. The collimated beam samples were irradiated on the same day as sample collection, and were plated in triplicate along with the flow test samples. Therefore, analytical conditions for the dose response data were also identical to those for the flow test samples.

The collimated beam results for MS2 are presented in Tables 4-2 and 4-3. These data were calculated as the average of the three individual results obtained at each dose level.

4.4 Development of Dose Response

The development of the UV dose response curves for MS2 for use with flow test data to establish the RED_{meas} is a three step process.

1. For each MS2 collimated beam test and its replicate for each day of testing, the log N (PFU/mL) was plotted vs. UV dose (mJ/cm²). Figures 4-1 and 4-2 show the curves for each day.
2. A separate equation (second order polynomial) was developed for each UVT condition (low and high). In this test there were two days of testing for MS2, so there were two sets of data. A common N_o was identified for each data set as the intercept of the curve at UV dose = 0.
3. The log inactivation (log I) was then calculated for each day for each measured value of N (including zero-dose) and the common N_o identified in Step 1 using the following equation:

$$\log I = \log(N_o/N)$$

Where:

N_o = The common N_o identified in Step 1 (PFU/mL); and

N = Concentration of challenge microorganisms in the petri dish after exposure to UV light (PFU/mL).

Tables 4-2 and 4-3 show the calculated values for MS2 log inactivation.

Finally, the UV dose as a function of log I was plotted for each day of the testing. Figures 4-3 through 4-4 show the curves for dose as a function of log I. Using regression analysis, an equation was derived that best fit the data, forcing the fit through the origin. The equation used for the dose response curve was a second order polynomial, which is the most common for MS2 collimated beam data. The regression equation was then used to calculate the RED_{meas} for each full scale flow test sample. RED_{meas} calculations and full scale data is presented in the Section 4.5.

A Grubb's test was run to determine if any replicates should be omitted from the development of the dose response curve. The Grubb's test results show that there was one outlier for replicate 2 for the high UVT water. The calculations were rerun after removing the outlier and that data was used for the subsequent determination of dose response and the RED calculations. Table 4-4 shows the data with the outlier removed. Both sets of data are presented for informational purposes. There is very little difference in the dose response curve coefficients between the data set with the outlier included when compared to the one with the outlier removed.

A summary of the statistics for uncertainty for the collimated beam dose response data is presented at the end of Tables 4-2, 4-3 and 4-4. The uncertainty (U_{DR}) of the collimated beam test was slightly higher than 30% at 1 log inactivation for the low UVT water (32.58%). The U_{DR} for the high UVT water was 12.25% once the outlier was removed (19.35% with the outlier included). At 2-log inactivation (dose of approximately 40 mJ/cm² RED) the U_{DR} was 15.31% and 5.53%. The uncertainty of the collimated beam results for Day 1 is greater than 30%. Therefore, the uncertainty calculations for the *Cryptosporidium* and *Giardia* log inactivation calculations presented in Sections 4.7 and 4.8 include the highest U_{DR} value (32.58%) applied to

all of the validation factors for the set points. The highest U_{DR} is also used for calculating the validation factor for calculating the RED_{val} based on MS2 as the target organism, as shown in Section 4.9.

Figures 4-3 through 4-4 show the results of the U_{DR} calculations plotted on the dose response curve. Also shown in Figures 4-3 and 4-4 are the QC limits for MS2 taken from the UVDGM-2006. The results show that the MS2 dose response curves are within the boundaries established for MS2.

The polynomial equation coefficients for each day (Aug 2 and 3, 2012) were evaluated statistically to determine which terms were statistically significant based on the P factor. All coefficients were found to be significant except for the x^2 term for the August 2, 2012 day of testing. The P factor was 0.0539, just above the 0.05 significance test cutoff. When P is greater than 0.05, it indicates the dose-response relationship could be linear rather than second order. Both linear and polynomial regressions were evaluated for this data set. The polynomial equation was selected for the RED_{meas} calculations because the R^2 coefficient was slightly better (0.996 versus 0.994), the standard error was slightly less (3.356 versus 3.755) and the other set of collimated beam data was a second order polynomial equation. Also the polynomial equation gave the more conservative (lower) results for the RED_{meas} . If the linear relationship is used, the RED_{meas} is approximately 6% higher than RED_{meas} calculated using the second order polynomial equation.

Table 4-2. UV Dose – Response Data from Collimated Beam Tests at 91% UVT

UVT (%)	Rep	Target UV Dose (mJ/cm ²)	Actual UV Dose	UV Dose ²	Avg (PFU/mL)	Avg Log(PFU/mL)	Log I	Log I ²	Predicted Dose	Residual (mJ/cm ²)	G	Outlier?
90.6	1	0	0.00	0	1,820,000	6.26	-0.02	0.000	-0.43	0.4	0.1	OK
		20	20.82	433	140,000	5.15	1.09	1.194	24.05	-3.2	1.0	OK
		30	31.25	977	80,300	4.90	1.33	1.780	29.81	1.4	0.4	OK
		40	41.54	1726	18,200	4.26	1.98	3.916	46.00	-4.5	1.4	OK
		60	62.27	3878	6,970	3.84	2.40	5.739	57.09	5.2	1.6	OK
		80	82.56	6816	707	2.85	3.39	11.488	85.49	-2.9	0.9	OK
	2	0	0.00	0	2,190,000	6.34	-0.10	0.010	-2.07	2.1	0.6	OK
		20	20.73	430	146,000	5.16	1.07	1.155	23.62	-2.9	0.9	OK
		30	31.12	968	84,700	4.93	1.31	1.719	29.25	1.9	0.6	OK
		40	41.37	1711	23,600	4.37	1.87	3.482	43.09	-1.7	0.6	OK
		60	62.05	3850	7,030	3.85	2.39	5.721	56.99	5.1	1.6	OK
		80	82.74	6846	873	2.94	3.30	10.876	82.75	0.0	0.0	OK

Avg:	0.07
SD:	3.20
n:	12
p:	0.05
t (95%):	2.228
Grubb's Test for Outliers	
p:	0.10
t (90%):	3.691
Grubb's Statistic (G _{CRIT}):	2.412

Table 4-2. (continued)

Uncertainty of Dose-Response (U_{DR})

Log I	Dose	t	SD	U_{DR} (%)	D_L (mJ/cm²/Log I)
0.001	0.0				20.48
0.25	5.2	2.23	3.20	136.89	20.83
0.50	10.6	2.23	3.20	67.31	21.18
1.00	21.9	2.23	3.20	32.58	21.88
1.50	33.9	2.23	3.20	21.05	22.58
2.00	46.6	2.23	3.20	15.31	23.28
2.50	59.9	2.23	3.20	11.89	23.98
3.00	74.0	2.23	3.20	9.63	24.68
3.50	88.8	2.23	3.20	8.03	25.38
4.00	104.3	2.23	3.20	6.83	26.08
3.39	85.5	2.23	3.20	8.34	25.22

t - student t test factor SD - standard deviation

Regression Statistics	
Multiple R	0.99796
R Square	0.995924
Adjusted R Square	0.895516
Standard Error	3.356386
Observations	12

ANOVA	df	SS	MS	F	Significance F
Regression	2	27522.43	13761.22	1221.555	1.09941E-11
Residual	10	112.6533	11.26533		
Total	12	27635.09			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	0							
X Variable 1	20.48157	1.751277	11.695219	3.72E-07	16.57948	24.38365	16.57948	24.38365272
X Variable 2	1.398414	0.640225	2.1842525	0.053857	-0.0281	2.824924	-0.0281	2.824924411

Table 4-3. UV Dose – Response Data from Collimated Beam Tests at 97% UVT

UVT (%)	Rep	Target UV Dose (mJ/cm ²)	Actual UV Dose	UV Dose ²	Avg (PFU/mL)	Avg Log(PFU/mL)	Log I	Log I ²	Predicted Dose	Residual (mJ/cm ²)	G	Outlier?
97.7	1	0	0.00	0	3,770,000	6.58	-0.06	0.004	-0.90	0.9	0.6	OK
		20	20.79	432	173,000	5.24	1.28	1.626	21.49	-0.7	0.5	OK
		30	31.09	967	74,300	4.87	1.64	2.696	28.83	2.3	1.5	OK
		40	41.34	1709	19,900	4.30	2.21	4.903	41.31	0.0	0.0	OK
		60	61.63	3798	3,070	3.49	3.03	9.156	61.17	0.5	0.3	OK
		80	81.83	6696	577	2.76	3.75	14.077	81.07	0.8	0.5	OK
	2	0	0.00	0	3,470,000	6.54	-0.03	0.001	-0.39	0.4	0.2	OK
		20	20.67	427	128,000	5.11	1.41	1.976	24.05	-3.4	2.4	OUTLIER
		30	30.85	952	70,000	4.85	1.67	2.782	29.37	1.5	1.0	OK
		40	41.05	1685	20,100	4.30	2.21	4.884	41.21	-0.2	0.2	OK
		60	61.40	3770	2,980	3.47	3.04	9.235	61.50	-0.1	0.1	OK
		80	81.78	6688	500	2.70	3.81	14.547	82.87	-1.1	0.8	OK
										Avg:	0.07	
										SD:	1.42	
										n:	12	
										p:	0.05	
										t (95%):	2.228	
										Grubb's Test for Outliers		
										p:	0.10	
										t (90%):	3.691	
										Grubb's Statistic (G _{CRIT}):	2.412	

Table 4-3. (continued)

Uncertainty of Dose-Response (U_{DR})

Log I	Dose	t	SD	U_{DR} (%)	D_L (mJ/cm²/Log I)
0.001	0.0				14.41
0.25	3.7	2.23	1.42	84.88	14.89
0.50	7.7	2.23	1.42	41.11	15.37
1.00	16.3	2.23	1.42	19.35	16.33
1.50	25.9	2.23	1.42	12.18	17.28
2.00	36.5	2.23	1.42	8.66	18.24
2.50	48.0	2.23	1.42	6.58	19.20
3.00	60.5	2.23	1.42	5.22	20.16
3.50	73.9	2.23	1.42	4.27	21.12
4.00	88.3	2.23	1.42	3.58	22.08
3.81	82.9	2.23	1.42	3.81	21.73

t - student t test factor SD - standard deviation

Regression Statistics	
Multiple R	0.999591
R Square	0.999183
Adjusted R Square	0.899101
Standard Error	1.488914
Observations	12

ANOVA	df	SS	MS	F	Significance F
Regression	2	27102.05	13551.02	6112.7	7.94E-15
Residual	10	22.16864	2.216864		
Total	12	27124.22			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	0							
X Variable 1	14.40581	0.708567	20.33092	1.83E-09	12.82703	15.9846	12.82703	15.9846
X Variable 2	1.919398	0.22588	8.497439	6.92E-06	1.416107	2.422689	1.416107	2.422689

Table 4-4. UV Dose – Response Data from Collimated Beam Tests at 97% UVT with outlier removed

UVT (%)	Rep	Target UV Dose (mJ/cm ²)	Actual UV Dose	UV Dose ²	Avg (PFU/mL)	Avg Log(PFU/mL)	Log I	Log I ²	Predicted Dose	Residual (mJ/cm ²)	G	Outlier?
97.7	1	0	0.00	0	3,770,000	6.58	-0.03	0.001	-0.49	0.5	0.5	OK
		20	20.79	432	173,000	5.24	1.30	1.702	22.16	-1.4	1.6	OK
		30	31.09	967	74,300	4.87	1.67	2.794	29.48	1.6	1.8	OK
		40	41.34	1709	19,900	4.30	2.24	5.034	41.85	-0.5	0.6	OK
		60	61.63	3798	3,070	3.49	3.06	9.335	61.39	0.2	0.2	OK
		80	81.83	6696	577	2.76	3.78	14.298	80.84	1.0	1.1	OK
	2	0	0.00	0	3,470,000	6.54	0.00	0.000	0.03	0.0	0.1	OK
		30	30.85	952	70,000	4.85	1.70	2.881	30.02	0.8	0.9	OK
		40	41.05	1685	20,100	4.30	2.24	5.014	41.75	-0.7	0.8	OK
		60	61.40	3770	2,980	3.47	3.07	9.414	61.72	-0.3	0.4	OK
		80	81.78	6688	500	2.70	3.84	14.773	82.59	-0.8	1.0	OK

Avg:	0.04
SD:	0.89
n:	11
p:	0.05
t (95%):	2.262
Grubb's Test for Outliers	
p:	0.10
t (90%):	3.751
Grubb's Statistic (G _{CRIT}):	2.355

Table 4-4. (continued)

Uncertainty of Dose-Response (U_{DR})

Log I	Dose	t	SD	U_{DR} (%)	D_L (mJ/cm²/Log I)
0.001	0.0				14.68
0.25	3.8	2.26	0.89	53.32	15.12
0.50	7.8	2.26	0.89	25.90	15.56
1.00	16.4	2.26	0.89	12.25	16.45
1.50	26.0	2.26	0.89	7.75	17.33
2.00	36.4	2.26	0.89	5.53	18.22
2.50	47.8	2.26	0.89	4.22	19.11
3.00	60.0	2.26	0.89	3.36	19.99
3.50	73.1	2.26	0.89	2.76	20.88
4.00	87.1	2.26	0.89	2.31	21.77
3.84	82.6	2.26	0.89	2.44	21.49

t - student t test factor SD - standard deviation

Regression Statistics	
Multiple R	0.999851
R Square	0.999702
Adjusted R Square	0.888558
Standard Error	0.940025
Observations	11

ANOVA	df	SS	MS	F	Significance F
Regression	2	26689.01	13344.51	15101.64	4.91681E-15
Residual	9	7.952815	0.883646		
Total	11	26696.97			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95%	Upper 95%
Intercept	0							
X Variable 1	14.67514	0.480973	30.511383	2.14E-10	13.5871	15.76317	13.5871	15.76317461
X Variable 2	1.772698	0.150326	11.792378	8.93E-07	1.432638	2.112759	1.432638	2.112758854

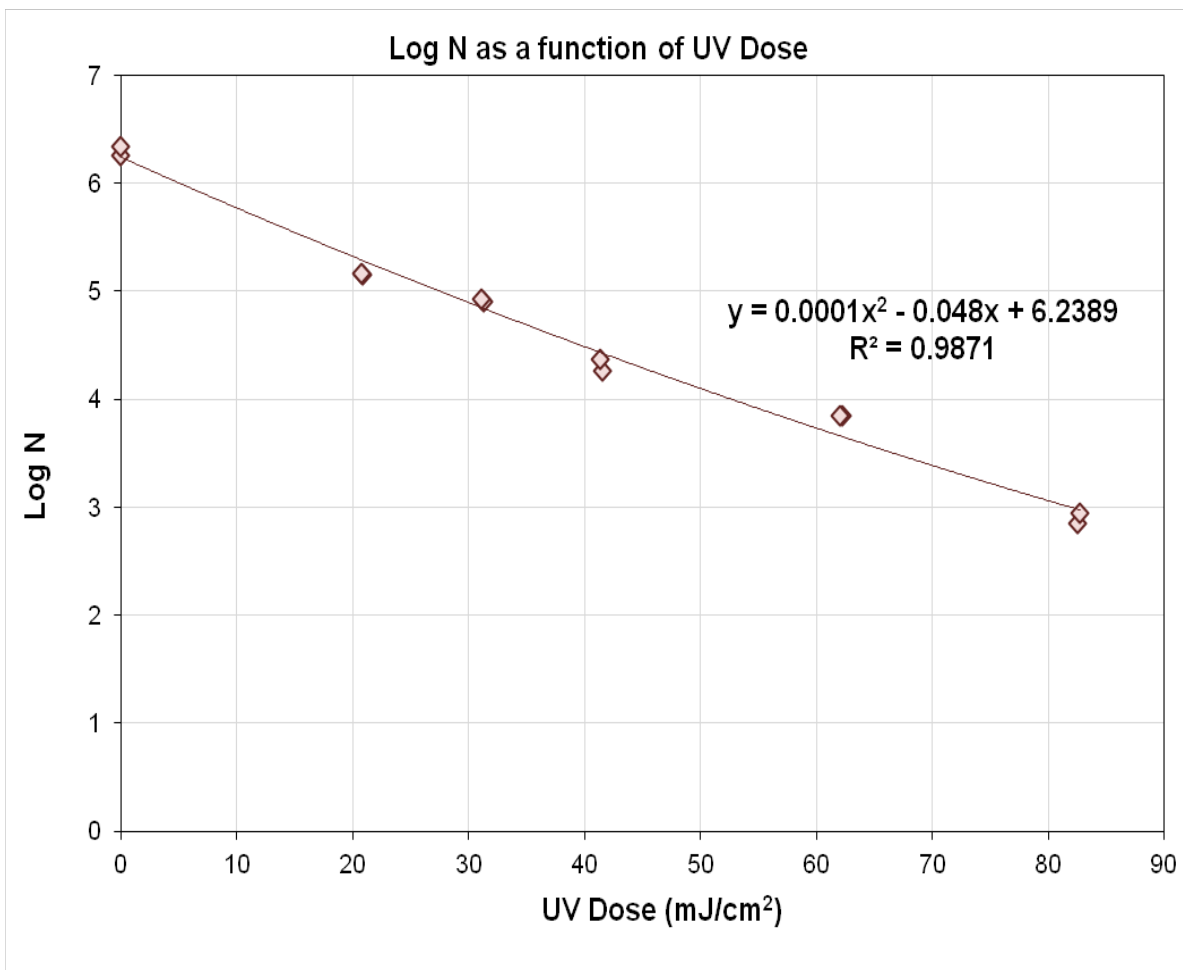


Figure 4-1. MS2 Collimated Beam Dose versus Log N Day 1 08-2-2012 UVT 91%.

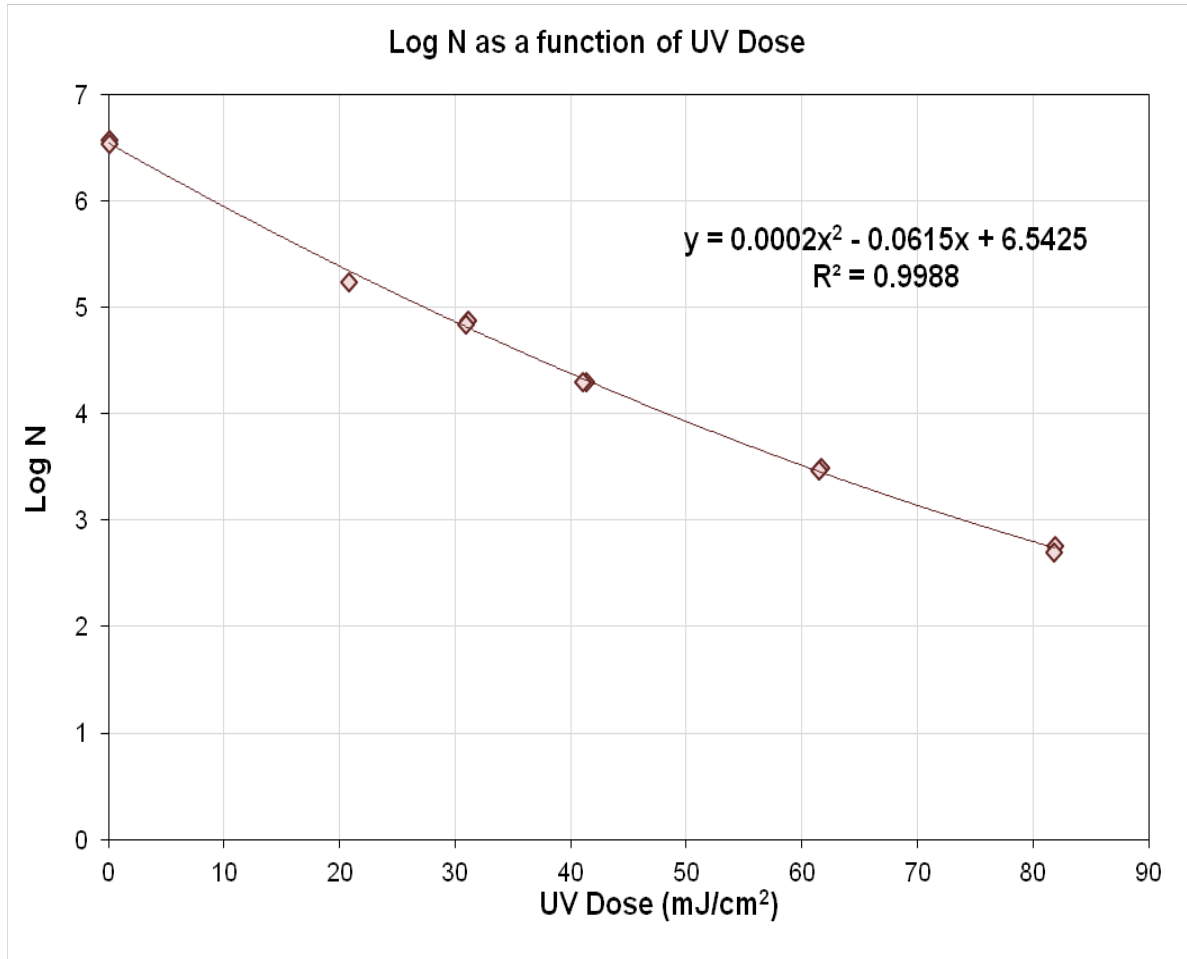


Figure 4-2. MS2 Collimated Beam Dose versus Log N Day 2 08-03-2012 97% UVT (outlier removed).

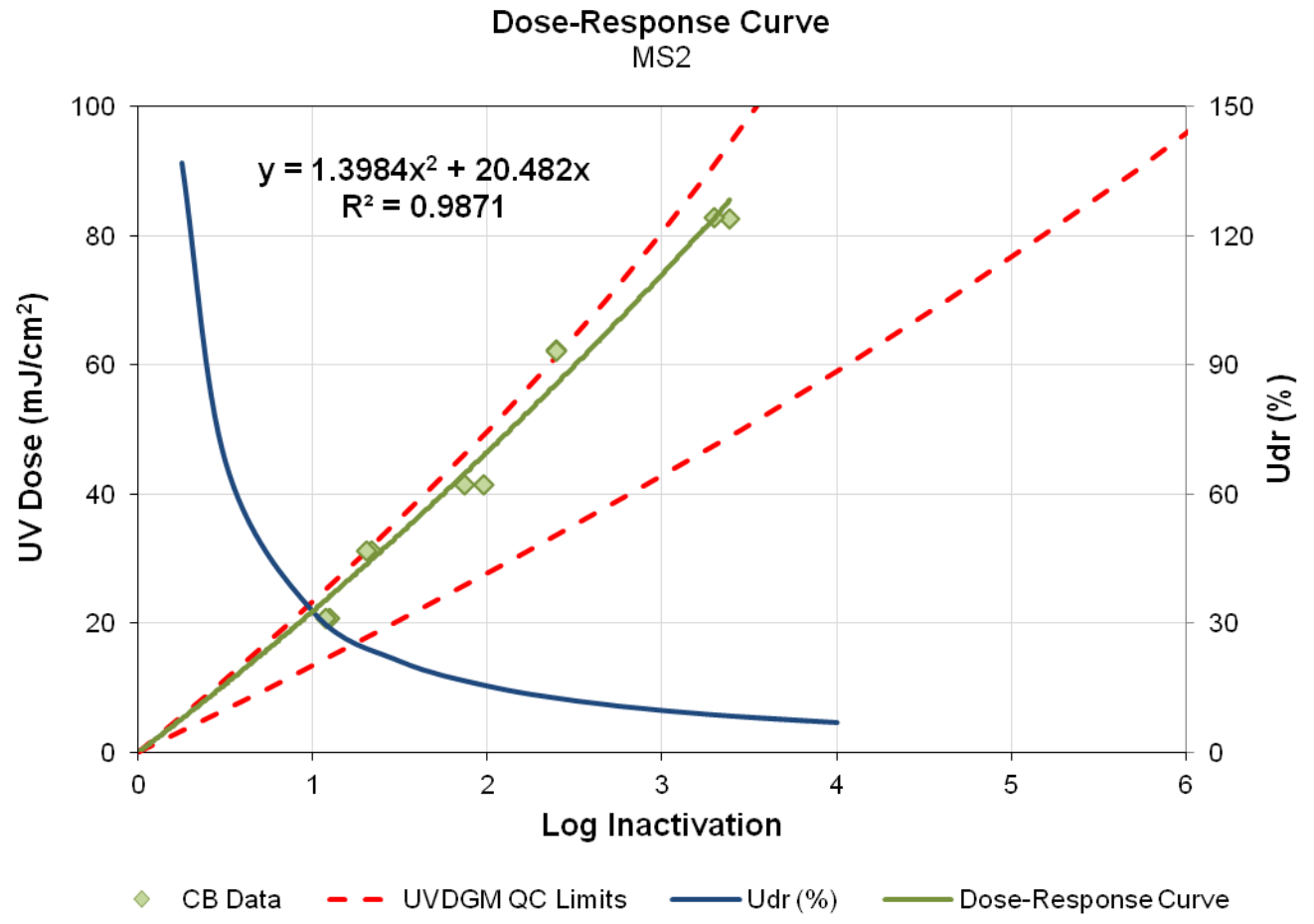


Figure 4-3. Dose response - log I versus dose -UVT 91% Day 1 08-02-2012.

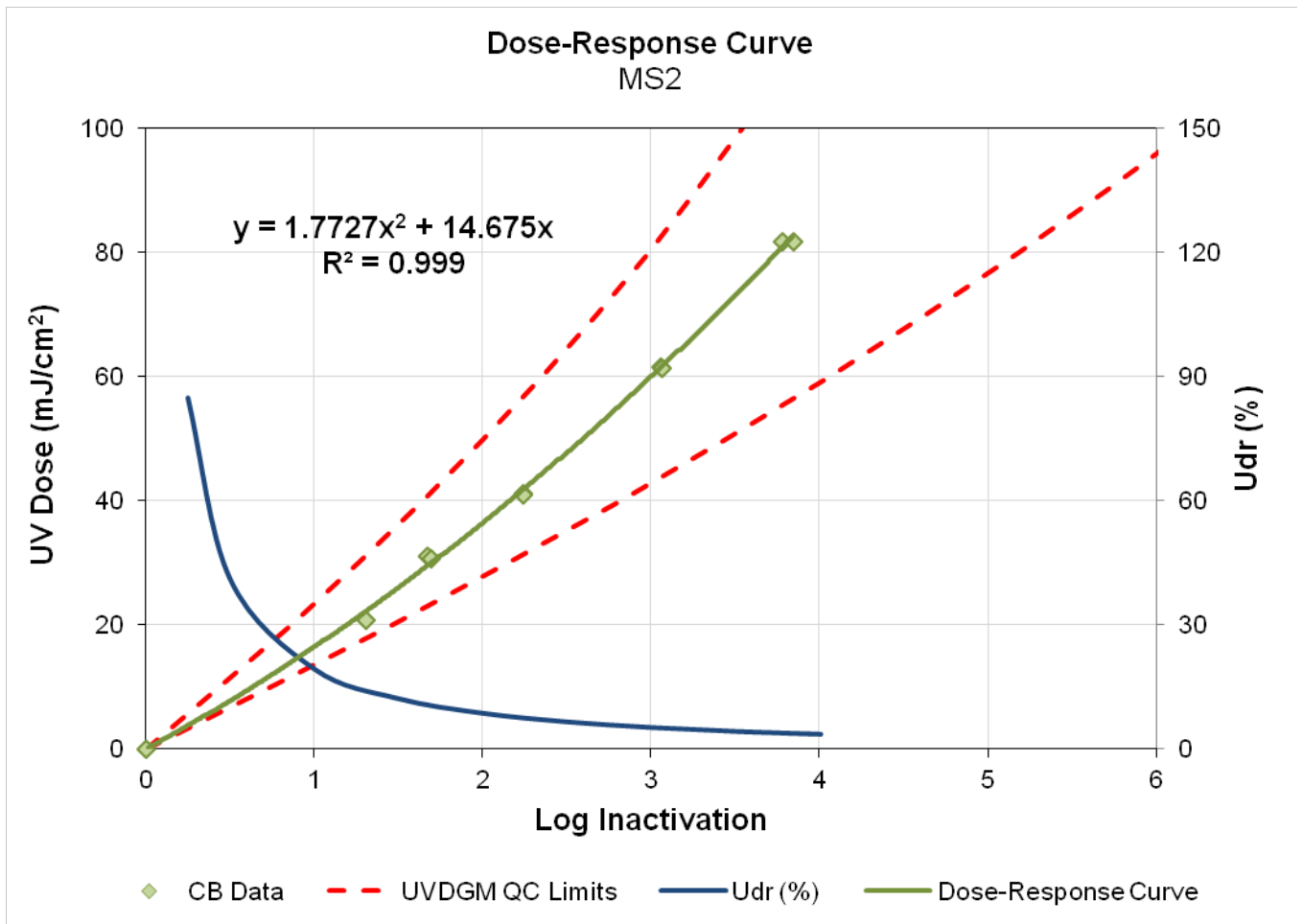


Figure 4-4. Dose response - log I versus dose -UVT 97% Day 2 08-03-2012 with outlier removed.

4.5 MS and Operational Flow Test Data

The operational data for each test run (flow rate, UVT, and UV sensor intensity measurements) are presented in Table 4-5. Flow rate, UVT and intensity were recorded when each sample was collected, thus providing five data points for each test run. These values were then used to obtain an average flow rate, UVT and intensity for each test run. The first influent and effluent samples for MS2 were taken simultaneously beginning after approximately 2 to 3 minutes of steady state operation. Subsequent influent and effluent samples were collected simultaneously after an additional 2 to 3 minutes of operation, yielding five sets of samples over a 10 to 12 minute period. The MS2 concentrations measured during the flow tests are presented in Table 4-6.

For each test condition replicate (i.e., each of the three influent and effluent samples), the log inactivation (log I) was calculated using the following equation:

$$\log I = \log (N_o / N)$$

Where:

N_o = challenge microorganism concentration in influent sample (PFU/mL); and
 N = Challenge microorganism concentration in corresponding effluent sample (PFU/mL).

Table 4-7 shows the log concentration for the influent (N_o) and effluent (N) samples. Table 4-8 presents the log I values for each sample.

The calculated RED_{meas} results in mJ/cm^2 are shown in Table 4-9. For each test condition the RED_{meas} for each replicate was determined using the measured log inactivation (log I) and the collimated beam test UV dose-response curve. The collimated beam regression line, based on the replicate collimated beam tests for each day of testing, was used for this calculation. Figures 4-3 and 4-4 present the dose response curves and the equations that were used to calculate the RED_{meas} .

The replicate RED_{meas} values were averaged to produce one RED_{meas} for each test condition and its duplicate. All of the flow tests at 150, 250, and 435 gpm, with feed water at 91%, 94%, and 97% UVT or the equivalent reduced power respectively, achieved a minimum RED_{meas} of 40 mJ/cm^2 .

Table 4-5. NeoTech D438™ Flow Test Operational Data

Test Condition	Test Day	Run	UVT (%)	Flow (gpm)	Intensity (mW/cm²)
Lowered UVT Full Power	1	2	91	151	7.9
Lowered UVT-Full Power Dup	1	3	91	151	7.9
High UVT - Lowered Power	2	10	98	155	7.9
High UVT - Lowered Power Dup	2	11	98	154	7.9
Lowered UVT Full Power	1	4	94	251	10.0
Lowered UVT-Full Power Dup	1	5	94	251	10.0
High UVT - Lowered Power	2	12	98	252	10.4
High UVT - Lowered Power Dup	2	13	98	252	10.4
Lowered UVT Full Power	1	6	97	436	13.0
Lowered UVT-Full Power Dup	1	7	96	436	13.0
High UVT - Lowered Power	2	14	98	434	13.1
High UVT - Lowered Power Dup	2	15	98	435	13.2

Table 4-6. Flow Tests MS2 Concentrations NeoTech D438™

Test Condition	Run	Influent (PFU/mL)					Effluent (PFU/mL)				
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Lowered UVT 91% Full Power	2	3.53E+05	4.53E+05	4.27E+05	4.27E+05	3.17E+05	1.24E+03	1.12E+03	1.10E+03	8.87E+02	7.80E+02
Lowered UVT 91% Full Power Dup	3	8.40E+05	5.60E+05	7.90E+05	7.37E+05	8.53E+05	4.37E+03	3.69E+03	4.22E+03	3.08E+03	3.11E+03
High UVT - Lowered Power	10	2.36E+06	1.18E+06	1.33E+06	1.15E+06	8.57E+05	1.40E+03	1.07E+03	1.19E+03	1.51E+03	1.31E+03
High UVT - Lowered Power Dup	11	1.07E+06	1.55E+06	9.10E+05	9.00E+05	8.07E+05	6.47E+02	6.23E+02	8.13E+02	8.67E+02	1.57E+03
Lowered UVT 94% Full Power	4	1.13E+06	8.73E+05	NA	8.97E+05	8.17E+05	3.88E+03	3.72E+03	NA	3.76E+03	4.64E+03
Lowered UVT 94% Full Power Dup	5	1.75E+06	1.59E+06	5.80E+05	6.20E+05	1.04E+06	4.20E+03	3.92E+03	4.00E+03	4.33E+03	4.43E+03
High UVT - Lowered Power	12	9.57E+05	9.60E+05	2.26E+06	1.59E+06	2.15E+06	6.27E+03	4.83E+03	5.30E+03	6.97E+03	7.50E+03
High UVT - Lowered Power Dup	13	1.81E+06	1.79E+06	2.24E+06	2.56E+06	2.04E+06	3.37E+03	4.10E+03	4.79E+03	3.13E+03	5.37E+03
Lowered UVT 97% Full Power	6	3.47E+06	1.56E+06	1.98E+06	2.00E+06	2.23E+06	6.33E+03	6.17E+03	5.33E+03	1.02E+04	7.50E+03
Lowered UVT 97% Full Power Dup	7	4.37E+06	5.60E+06	2.00E+06	1.78E+06	2.31E+06	2.28E+04	1.18E+04	1.42E+04	1.63E+04	1.53E+04
High UVT - Lowered Power	14	3.18E+06	2.05E+06	1.94E+06	1.57E+06	2.49E+06	9.43E+03	1.25E+04	5.60E+03	6.40E+03	1.13E+04
High UVT - Lowered Power Dup	15	2.97E+06	2.90E+06	3.81E+06	2.86E+06	3.38E+06	1.08E+04	1.50E+04	7.30E+03	1.17E+04	7.40E+03

NA- samples not analyzed

Table 4-7. MS2 Log Concentration for Influent and Effluent Samples NeoTech D438™

Test Condition	Run	Log Influent Concentration					Log Effluent Concentration				
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Lowered UVT 91% Full Power	2	5.55	5.66	5.63	5.63	5.50	3.09	3.05	3.04	2.95	2.89
Lowered UVT 91% Full Power Dup	3	5.92	5.75	5.90	5.87	5.93	3.64	3.57	3.63	3.49	3.49
High UVT - Lowered Power	10	6.37	6.07	6.12	6.06	5.93	3.15	3.03	3.08	3.18	3.12
High UVT - Lowered Power Dup	11	6.03	6.19	5.96	5.95	5.91	2.81	2.79	2.91	2.94	3.20
Lowered UVT 94% Full Power	4	6.05	5.94	NA	5.95	5.91	3.59	3.57	NA	3.58	3.67
Lowered UVT 94% Full Power Dup	5	6.24	6.20	5.76	5.79	6.02	3.62	3.59	3.60	3.64	3.65
High UVT - Lowered Power	12	5.98	5.98	6.35	6.20	6.33	3.80	3.68	3.72	3.84	3.88
High UVT - Lowered Power Dup	13	6.26	6.25	6.35	6.41	6.31	3.53	3.61	3.68	3.50	3.73
Lowered UVT 97% Full Power	6	6.54	6.19	6.30	6.30	6.35	3.80	3.79	3.73	4.01	3.88
Lowered UVT 97% Full Power Dup	7	6.64	6.75	6.30	6.25	6.36	4.36	4.07	4.15	4.21	4.18
High UVT - Lowered Power	14	6.50	6.31	6.29	6.20	6.40	3.97	4.10	3.75	3.81	4.05
High UVT - Lowered Power Dup	15	6.47	6.46	6.58	6.46	6.53	4.03	4.18	3.86	4.07	3.87

NA- samples not analyzed

Table 4-8. MS2 Log Inactivation Results NeoTech D438™

Test Condition	Test Run #	Test Day	UVT (%)	Flow (gpm)	Intensity (mW/cm ²)	Log I				
						Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Lowered UVT 91% Full Power	2	1	90.6	151.1	7.9	2.45	2.61	2.59	2.68	2.61
Lowered UVT 91% Full Power Dup	3	1	90.6	151.3	7.9	2.28	2.18	2.27	2.38	2.44
High UVT - Lowered Power	10	2	98.1	154.8	7.9	3.23	3.04	3.05	2.88	2.82
High UVT - Lowered Power Dup	11	2	98.1	154.1	7.9	3.22	3.40	3.05	3.02	2.71
Lowered UVT 94% Full Power	4	1	93.9	251.4	10.0	2.46	2.37	NA	2.38	2.25
Lowered UVT 94% Full Power Dup	5	1	93.9	250.8	10.0	2.62	2.61	2.16	2.16	2.37
High UVT - Lowered Power	12	2	98.2	251.8	10.4	2.18	2.30	2.63	2.36	2.46
High UVT - Lowered Power Dup	13	2	98.2	251.6	10.4	2.73	2.64	2.67	2.91	2.58
Lowered UVT 97% Full Power	6	1	96.5	436.0	13.0	2.74	2.40	2.57	2.29	2.47
Lowered UVT 97% Full Power Dup	7	1	96.4	435.5	13.0	2.28	2.68	2.15	2.04	2.18
High UVT - Lowered Power	14	2	98.1	434.2	13.1	2.53	2.21	2.54	2.39	2.34
High UVT - Lowered Power Dup	15	2	98.2	435.8	13.2	2.44	2.29	2.72	2.39	2.66

NA- samples not analyzed

Table 4-9. MS2 RED Results NeoTech D438™

Test Condition	Test Run	Test Day	UVT (%)	Flow (gpm)	Intensity (mW/cm ²)	RED (mJ/cm ²)						SD(RED)	U _{SP}
						Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average		
Lowered UVT 91%	2	1	90.6	151	7.9	58.69	62.90	62.40	65.01	62.96	62.39	2.30	10.21
Lowered UVT 91% Dup	3	1	90.6	151	7.9	54.07	51.33	53.76	56.64	58.25	54.81	2.69	13.63
Lowered Power	10	2	98.1	155	7.9	65.81	61.06	61.21	57.01	55.37	60.09	4.08	18.85
Lowered Power Dup	11	2	98.1	154	7.9	65.59	70.28	61.22	60.39	52.81	62.06	6.50	29.08
Lowered UVT 94%	4	1	93.9	251	10.0	58.96	56.41	NA	56.60	53.05	56.26	2.43	13.76
Lowered UVT 94% Dup	5	1	93.9	251	10.0	63.26	62.93	50.80	50.66	56.41	56.81	6.19	30.23
Lowered Power	12	2	98.2	252	10.4	40.50	43.09	50.85	44.46	46.77	45.13	3.92	24.11
Lowered Power Dup	13	2	98.2	252	10.4	53.28	51.10	51.82	57.78	49.65	52.73	3.11	16.40
Lowered UVT 97%	6	1	96.5	436	13.0	66.59	57.29	61.87	54.30	59.21	59.85	4.67	21.66
Lowered UVT 97% Dup	7	1	96.4	436	13.0	54.04	64.83	50.47	47.56	51.27	53.63	6.67	34.54
Lowered Power	14	2	98.1	434	13.1	48.43	41.20	48.70	45.19	44.12	45.53	3.13	19.12
Lowered Power Dup	15	2	98.2	436	13.2	46.35	42.82	52.97	45.16	51.57	47.77	4.33	25.14

NA- not analyzed

4.6 Set Line for RED_{meas} of 40 mJ/cm^2

The three set point conditions selected for this validation all achieved a minimum RED_{meas} of 40 mJ/cm^2 , which was the target minimum RED_{meas} for developing the set line. Figure 4-5 shows the set line. The unit is validated for a minimum RED_{meas} of 40 mJ/cm^2 for any flow rate - intensity combination above and to the left of the set line. The maximum flow rate demonstrated was 434 gpm. A UV system cannot operate above the highest validated flow rate and claim a 40 mJ/cm^2 RED_{meas} . The lowest intensity demonstrating a RED of 40 mJ/cm^2 was 7.9 mW/cm^2 . A UV system cannot operate below the lowest validated irradiance and claim a RED_{meas} of 40 mJ/cm^2 .

Set Point 1 - 151 gpm; 7.9 mW/cm^2

Set Point 2 - 251 gpm; 10.4 mW/cm^2

Set Point 3 - 434 gpm; 13.2 mW/cm^2

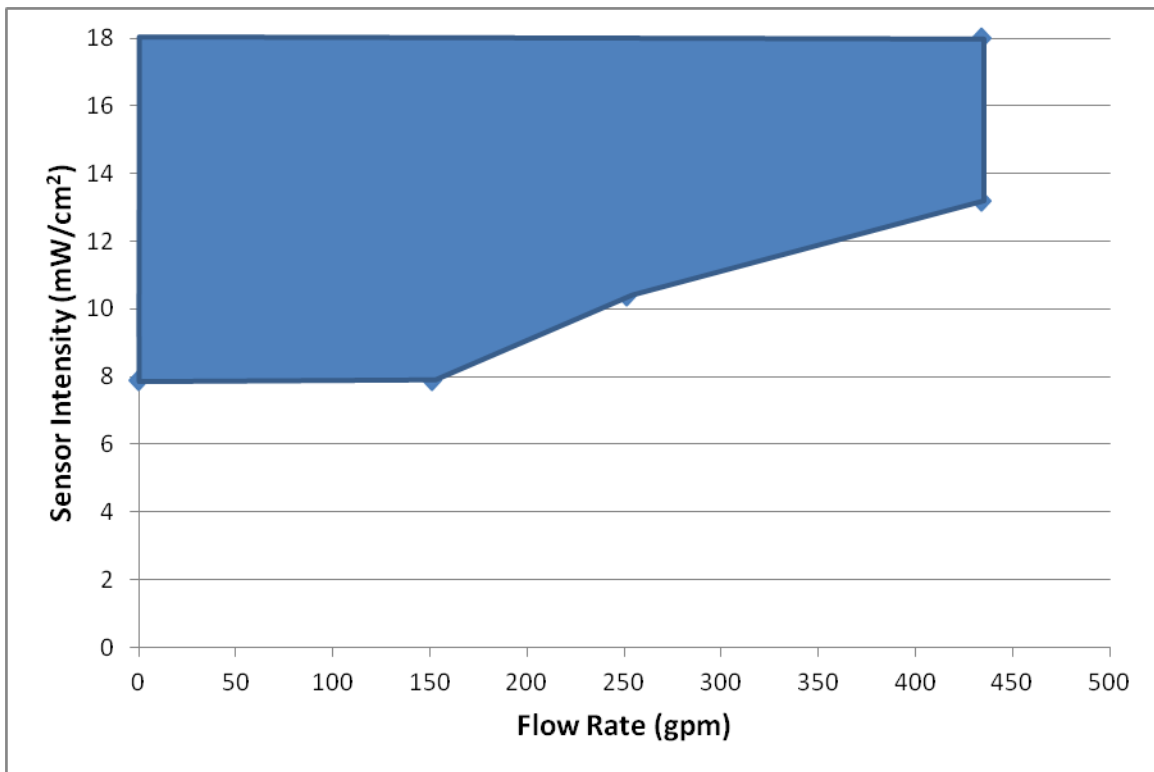


Figure 4-5. Set Line for 40 mJ/cm^2 RED_{meas} - NeoTech D438™.

4.7 Deriving the Validation Factor and Log Inactivation for *Cryptosporidium*

4.7.1 Validation Factor Definition

Several uncertainties and biases are involved in using experimental testing to define a validated dose and validated operating conditions such as challenge microorganism UV sensitivity, and sensor placement or variability. The validation factor (VF) for *Cryptosporidium* was determined quantitatively to account for key areas of uncertainty and variability. The equation for the VF is shown below.

$$VF = B_{RED} \times [1 + (U_{Val} / 100)]$$

where:

VF = Validation Factor;

B_{RED} = RED bias factor;

U_{Val} = Uncertainty of validation expressed as a percentage.

The data used for the VF calculations and final results are presented in the following section.

4.7.2 RED Bias (B_{RED})

The RED bias factor (B_{RED}) is a correction factor that accounts for the difference between the UV sensitivity of a selected target pathogen and the UV sensitivity of the challenge microorganism (MS2). If the challenge microorganism is more resistant (less sensitive) to UV light than the target pathogen, the RED measured during the validation will be greater than the RED that would be measured for the target pathogen. In this case the RED bias would be greater than 1.0. If the challenge microorganism is less resistant (more sensitive) to UV light than the target pathogen, then RED measured by the validation will be less than the RED that would be measured for the target pathogen.

A target pathogen must be selected to calculate the RED bias factor. For this test, the target pathogen *Cryptosporidium* was selected for use in presenting an example calculation of RED bias as it is a common pathogen that is evaluated for drinking water applications. *Cryptosporidium* was also selected because the EPA's LT2ESWTR requires UV reactors be validated to demonstrate a log inactivation for *Cryptosporidium*. A target of 3-log inactivation of *Cryptosporidium* was selected as water utilities in the highest risk category or "bin" may need this maximum level of inactivation. The RED bias tables in Appendix G of the UVDGM-2006 were used for determining the RED bias. The RED bias is determined from the Tables based on the sensitivity calculated for each test run replicate at a given set point (test condition) and the UVT of the water. Sensitivity is calculated as:

$$\text{Sensitivity (mJ/cm}^2 \text{ per log I)} = \text{RED} / \text{Log I}$$

Per the GP-2011 and UVDGM-2006, the sensitivity is calculated for each test replicate (five per test run, 20 samples total per set point). The highest B_{RED} value found among the replicates at a given set point is then selected for the B_{RED} value for use in the VF calculation per the UVDGM-2006 requirement. Table 4-10 shows the data for the replicates at each set point. The highest

RED bias at each set point is used in the validation factor calculations shown later in Section 4.7.4.

Table 4-10. RED Bias Factor for Each Set Point for *Cryptosporidium*

Sample Number	Test Run	UVT %	Sensitivity mJ/cm ² per Log I			B _{RED} 4-log <i>Crypto</i>	B _{RED} 3.5-log <i>Crypto</i>	B _{RED} 3.0 log <i>Crypto</i>
			RED	Log I	Sensitivity			
2-1	2	90.6	58.69	2.45	23.91	1.66	1.79	1.82
2-2	2	90.6	62.90	2.61	24.13	1.70	1.83	1.85
2-3	2	90.6	62.40	2.59	24.10	1.70	1.83	1.85
2-4	2	90.6	65.01	2.68	24.23	1.70	1.83	1.85
2-5	2	90.6	62.96	2.61	24.13	1.70	1.83	1.85
3-1	3	90.6	54.07	2.28	23.68	1.66	1.79	1.82
3-2	3	90.6	51.33	2.18	23.53	1.66	1.79	1.82
3-3	3	90.6	53.76	2.27	23.66	1.66	1.79	1.82
3-4	3	90.6	56.64	2.38	23.81	1.66	1.79	1.82
3-5	3	90.6	58.25	2.44	23.89	1.66	1.79	1.82
4-1	4	93.9	58.96	2.46	23.93	1.66	1.79	1.82
4-2	4	93.9	56.41	2.37	23.80	1.66	1.79	1.82
4-3	4	93.9	NA	NA	NA	NA	NA	NA
4-4	4	93.9	56.60	2.38	23.81	1.66	1.79	1.82
4-5	4	93.9	53.05	2.25	23.62	1.66	1.79	1.82
5-1	5	93.9	63.26	2.62	24.15	1.70	1.83	1.85
5-2	5	93.9	62.93	2.61	24.13	1.70	1.83	1.85
5-3	5	93.9	50.80	2.16	23.50	1.66	1.79	1.82
5-4	5	93.9	50.66	2.16	23.50	1.66	1.79	1.82
5-5	5	93.9	56.41	2.37	23.80	1.66	1.79	1.82
6-1	6	96.5	66.59	2.74	24.31	1.40	1.43	1.42
6-2	6	96.5	57.29	2.40	23.84	1.38	1.42	1.41
6-3	6	96.5	61.87	2.57	24.08	1.40	1.43	1.42
6-4	6	96.5	54.30	2.29	23.69	1.38	1.42	1.41
6-5	6	96.5	59.21	2.47	23.94	1.38	1.42	1.41
7-1	7	96.4	54.04	2.28	23.67	1.38	1.42	1.41
7-2	7	96.4	64.83	2.68	24.22	1.40	1.43	1.42
7-3	7	96.4	50.47	2.15	23.49	1.38	1.42	1.41
7-4	7	96.4	47.56	2.04	23.33	1.38	1.42	1.41
7-5	7	96.4	51.27	2.18	23.53	1.38	1.42	1.41
10-1	10	98.1	65.81	3.23	20.40	1.18	1.20	1.20
10-1	10	98.1	61.06	3.04	20.07	1.18	1.20	1.20
10-1	10	98.1	61.21	3.05	20.08	1.18	1.20	1.20
10-1	10	98.1	57.01	2.88	19.78	1.17	1.19	1.19
10-1	10	98.1	55.37	2.82	19.67	1.17	1.19	1.19
11-1	11	98.1	65.59	3.22	20.38	1.18	1.20	1.20
11-2	11	98.1	70.28	3.40	20.69	1.18	1.20	1.20
11-3	11	98.1	61.22	3.05	20.08	1.18	1.20	1.20
11-4	11	98.1	60.39	3.02	20.02	1.18	1.20	1.20
11-5	11	98.1	52.81	2.71	19.48	1.17	1.19	1.19
12-1	12	98.2	40.50	2.18	18.55	1.17	1.19	1.19
12-2	12	98.2	43.09	2.30	18.75	1.17	1.19	1.19

Sample Number	Test Run	UVT %	Sensitivity mJ/cm ² per Log I			B _{RED} 4-log <i>Crypto</i>	B _{RED} 3.5-log <i>Crypto</i>	B _{RED} 3.0 log <i>Crypto</i>
			RED	Log I	Sensitivity			
12-3	12	98.2	50.85	2.63	19.34	1.17	1.19	1.19
12-4	12	98.2	44.46	2.36	18.86	1.17	1.19	1.19
12-5	12	98.2	46.77	2.46	19.03	1.17	1.19	1.19
13-1	13	98.2	53.28	2.73	19.51	1.17	1.19	1.19
13-2	13	98.2	51.10	2.64	19.36	1.17	1.19	1.19
13-3	13	98.2	51.82	2.67	19.41	1.17	1.19	1.19
13-4	13	98.2	57.78	2.91	19.84	1.17	1.19	1.19
13-5	13	98.2	49.65	2.58	19.25	1.17	1.19	1.19
14-1	14	98.1	48.43	2.53	19.16	1.17	1.19	1.19
14-2	14	98.1	41.20	2.21	18.60	1.17	1.19	1.19
14-3	14	98.1	48.70	2.54	19.18	1.17	1.19	1.19
14-4	14	98.1	45.19	2.39	18.91	1.17	1.19	1.19
14-5	14	98.1	44.12	2.34	18.83	1.17	1.19	1.19
15-1	15	98.2	46.35	2.44	19.00	1.17	1.19	1.19
15-2	15	98.2	42.82	2.29	18.73	1.17	1.19	1.19
15-3	15	98.2	52.97	2.72	19.49	1.17	1.19	1.19
15-4	15	98.2	45.16	2.39	18.91	1.17	1.19	1.19
15-5	15	98.2	51.57	2.66	19.39	1.17	1.19	1.19
Maximum B_{RED}	Set Point 151 gpm - 7.9 mW/cm ²					1.70	1.83	1.85
	Set Point 251 gpm - 10.4 mW/cm ²					1.70	1.83	1.85
	Set Point 434 gpm - 13.2 mW/cm ²					1.40	1.43	1.42

NA - sample not analyzed so RED and bias not determined

Uncertainty of Validation

The uncertainty of validation (U_{Val}) addresses many sources of experimental uncertainty. As the critical source of uncertainty, such as the sensor readings, or the fit of the dose-response curve, is unknown in advance of the validation testing, the USEPA developed a decision tree to assist in establishing U_{Val} . Figure 5.4 of the UVDGM-2006, which is specific to a UV intensity set point approach, was used to determine U_{Val} in calculating the validated dose. Per Figure 5.4 in EPA's UVDGM-2006, any of the following equations may be used to establish the U_{Val} :

$$U_{Val} = (U_{SP}^2 + U_S^2)^{1/2}$$

$$U_{Val} = U_{SP}$$

$$U_{Val} = (U_{SP}^2 + U_{DR}^2)^{1/2}$$

$$U_{Val} = (U_{SP}^2 + U_S^2 + U_{DR}^2)^{1/2}$$

Where:

U_S = Uncertainty of sensor value, expressed as a fraction;

U_{DR} = Uncertainty of the fit of the dose-response curve;

U_{SP} = Uncertainty of set-point; and

U_{Val} = Uncertainty of the validation.

The QC objective for the duty sensor is that the measurements with the duty sensor should be $\leq 10\%$ of the average of two or more reference sensors. If this objective is met, then it eliminates

the need to calculate the U_S factor per the GP-2011 and UVDGM-2006, Section 5.4.4. The sensor met the 10% requirement, as shown in Table 4-1, therefore, U_S is not used in determining the uncertainty of validation.

The UVDGM-2006 also shows the formula and calculations for U_{DR} in Appendix C Section C4.

The equation is:

$$U_{DR} = t * [SD / UV Dose_{CB}] * 100\%$$

Where:

U_{DR} = Uncertainty of the UV dose-response fit at a 95% confidence level;

$UV Dose_{CB}$ = UV dose calculated from the UV dose-response curve for the challenge microorganism;

SD = Standard deviation of the difference between the calculated UV dose response and the measured value; and

t = t-statistic at a 95% confidence level for a sample size equal to the number of test condition replicates used to define the dose-response.

The U_{DR} results are shown in Tables 4-2 and 4-4 for the low and high UVT waters (32.58% and 12.25%, respectively). Since the U_{DR} was $> 30\%$ at the UV dose corresponding to 1-log inactivation of the challenge organism, the uncertainty of the dose response (U_{DR}) is included in the calculation of uncertainty.

The uncertainty in the set point value (U_{SP}) is based on a prediction interval at a 95% confidence level using the following procedure:

1. Calculate the average and standard deviation of RED_{meas} values for each test condition
2. Calculate the uncertainty of the set point RED_{meas} using:

$$U_{SP} = [(t \times SD_{RED}) / (RED_{meas})] \times 100\%$$

Where:

RED_{meas} = Average RED_{meas} value measured for each test condition;

SD_{RED} = Standard deviation of the RED_{meas} values measured for each test condition; and

t = t-statistic for a 95% confidence level defined as a function of the number of replicate samples, in this case 5 replicates were used for testing yielding a t value of 2.776 ($n-1 = 4$), except run 4 which had four valid replicates so the t value is 3.182).

3. Select the highest U_{SP} from the replicates at each set point for calculating the VF.

The U_{SP} results based on the RED_{meas} and standard deviation are shown in Table 4-9. The highest U_{SP} for each set point is 29.08% (151 gpm set point), 30.23% (251 gpm set point), and 34.54% (434 gpm set point).

The uncertainty of the validation is then calculated using the highest U_{DR} (32.58%) from the 2012 dose response data and the highest U_{SP} for each test condition using the equation:

$$U_{Val} = (U_{SP}^2 + U_{DR}^2)^{1/2}$$

Table 4-11 shows the U_{Val} values used for determining the uncertainty of the validation at each set point.

Table 4-11. Uncertainty of the Validation (U_{Val}) and B_{RED} Values for *Cryptosporidium*

Set Point	Max U_{DR} (%)	Max U_{SP} (%)	U_{Val} (%)	Max B_{RED}		
				4.0-log	3.5-log	3.0-log
151 gpm - 7.9 mW/cm ²	32.58	29.08	43.67	1.70	1.83	1.85
251 gpm - 10.4 mW/cm ²	32.58	30.23	44.44	1.70	1.83	1.85
434 gpm - 13.2 mW/cm ²	32.58	34.54	47.48	1.40	1.43	1.42

4.7.4 Validated Dose and Set Line for *Cryptosporidium*

After establishing the U_{Val} and the RED bias as described above, the VF is calculated using the equation:

$$VF = B_{RED} \times [1 + (U_{Val} / 100)]$$

Where:

VF = Validation Factor;

B_{RED} = RED bias factor for *Cryptosporidium*; and

U_{Val} = Uncertainty of validation expressed as a percentage.

The validated dose is then calculated as follows:

$$\text{Validated dose } (RED_{Val}) = RED_{meas} / VF$$

Table 4-12 shows the calculated VF for various *Cryptosporidium* log inactivation levels (3.0, 3.5, and 4.0 log inactivation).

Table 4-12 shows the RED_{Val} for *Cryptosporidium* for each test run using the validation factors for the various *Cryptosporidium* log inactivation levels. Table 4-12 also shows the Validated Dose for each set point and a comparison to the dose required for various levels of inactivation of *Cryptosporidium*. As can be seen, the tests for the first and third set points (151 and 434 gpm) show a validated dose for *Cryptosporidium* that would achieve a minimum of 4.0 log inactivation. The second set point (middle point) at 251 gpm showed a minimum of 3.5 log inactivation.

The NeoTech D438™ achieved a minimum of 3.5 log inactivation for the low power runs at 251 gpm at 10.4 mW/cm² and a 4.0-log inactivation for all of the test runs at the set points at 151 gpm - 7.9 mW/cm² and 434 gpm - 13.2 mW/cm². Figure 4-6 shows the operating conditions based on three set points that achieved the 3-log inactivation of *Cryptosporidium*.

The NeoTech D438™ achieved a minimum 3.0-log inactivation for *Cryptosporidium* over the range of flow (151 to 434 gpm) and intensity (7.9 to 13.2 mW/cm²) as shown in the set line in Figure 4-14.

The set points in Figure 4-6 are:

- Set Point 1 - 151 gpm; 7.9 mW/cm²
- Set Point 2 - 251 gpm; 10.4 mW/cm²
- Set Point 3 - 434 gpm; 13.2 mW/cm²

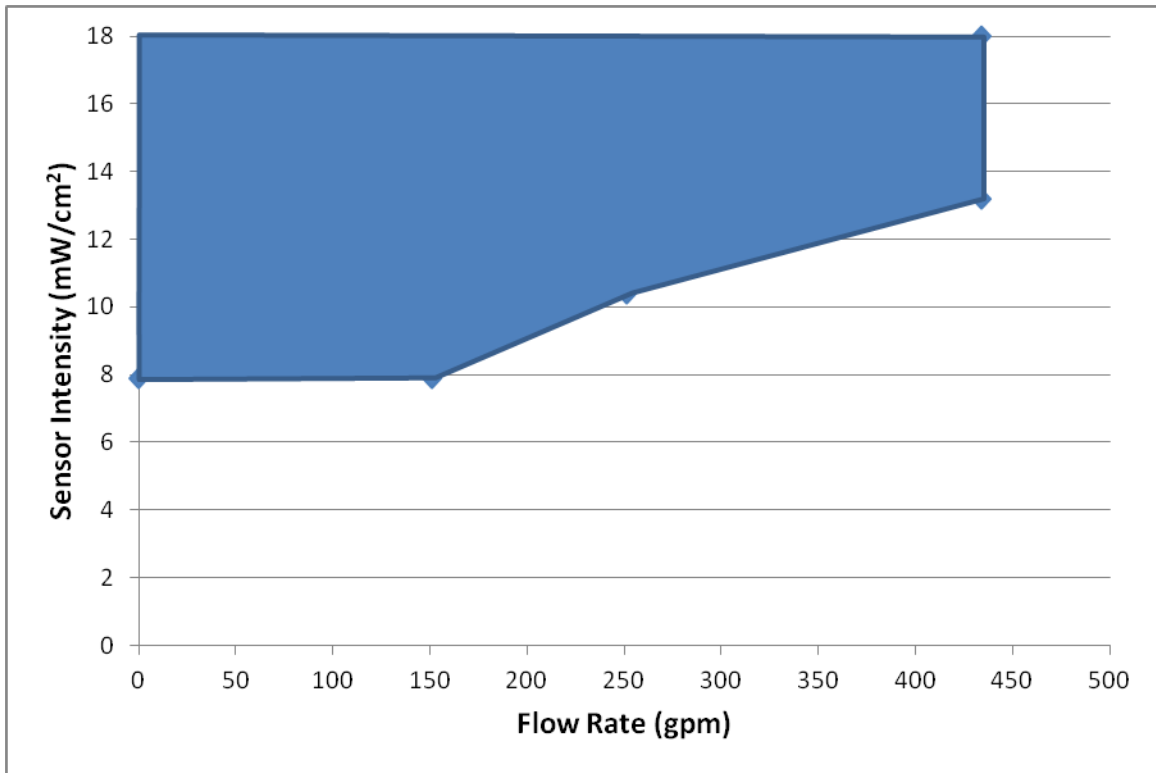


Figure 4-6. Set Line for Minimum 3-log *Cryptosporidium* Inactivation for NeoTech D438™.

Table 4-12. Validation Factors and Validated Dose (RED_{Val}) for *Cryptosporidium*

Condition	Run #	Flow Rate (gpm)	Intensity (mW/cm ²)	Validation Factor			RED _{meas} (mJ/cm ²)	RED _{Val}		
				4.0-log	3.5-log	3.0-log		4-log (mJ/cm ²)	3.5-log (mJ/cm ²)	3.0-log (mJ/cm ²)
								22 ⁽¹⁾	15 ⁽¹⁾	12 ⁽¹⁾
Lowered UVT	2	151.1	7.9	2.44	2.63	2.66	62.39	25.5	23.7	23.5
Lowered UVT Dup	3	151.3	7.9	2.44	2.63	2.66	54.81	22.4	20.8	20.6
Lowered Power	10	154.8	7.9	2.44	2.63	2.66	60.09	24.6	22.9	22.6
Lowered Power Dup	11	154.1	7.9	2.44	2.63	2.66	62.06	25.4	23.6	23.3
Lowered UVT	4	251.4	10.0	2.46	2.64	2.67	56.26	22.9	21.3	21.1
Lowered UVT Dup	5	250.8	10.0	2.46	2.64	2.67	56.81	23.1	21.5	21.3
Lowered Power	12	251.8	10.4	2.46	2.64	2.67	45.13	18.4	17.1	16.9
Lowered Power Dup	13	251.6	10.4	2.46	2.64	2.67	52.73	21.5	19.9	19.7
Lowered UVT	6	436.0	13.0	2.06	2.11	2.09	59.85	29.0	28.4	28.6
Lowered UVT Dup	7	435.5	13.0	2.06	2.11	2.09	53.63	26.0	25.4	25.6
Lowered Power	14	434.2	13.1	2.06	2.11	2.09	45.53	22.1	21.6	21.7
Lowered Power Dup	15	435.8	13.2	2.06	2.11	2.09	47.77	23.1	22.7	22.8

(1) Required dose for log inactivation validation per the UVDGM-2006 Appendix G.

4.8 Deriving the Validation Factor and Log Inactivation for *Giardia*

4.8.1 Validation Factor Definition

As described earlier in Section 4.7.1 on *Cryptosporidium*, several uncertainties and biases are involved in using experimental testing to define a validated dose and validated operating conditions such as challenge microorganism UV sensitivity, and sensor placement or variability. The VF for *Giardia* was determined quantitatively to account for key areas of uncertainty and variability. The equation for the VF is shown below.

$$VF = B_{RED} \times [1 + (U_{Val} / 100)]$$

where:

VF = Validation Factor;

B_{RED} = RED bias factor;

U_{Val} = Uncertainty of validation expressed as a percentage.

The data used for the VF calculations and final results are presented below.

4.8.2 RED Bias (B_{RED})

The RED bias factor (B_{RED}) is a correction factor that accounts for the difference between the UV sensitivity of a selected target pathogen and the UV sensitivity of the challenge microorganism (MS2). As described in Section 4.7.2, a target pathogen must be selected to calculate the RED bias factor. In addition to the target pathogen *Cryptosporidium* described previously, *Giardia* was also selected for evaluation. The RED bias tables for *Giardia* in Appendix G of the UVDGM-2006 were used for determining the RED bias. The RED bias is determined from the Tables based on the sensitivity calculated for each test run replicate at a given set point (test condition) and the UVT of the water. Sensitivity is calculated as:

$$\text{Sensitivity (mJ/cm}^2 \text{ per log I)} = \text{RED} / \text{Log I}$$

Per the GP-2011 and UVDGM-2006, the sensitivity is calculated for each test replicate (five per test run, 20 samples total per set point). The highest B_{RED} value found among the replicates at a given set point is then selected for the B_{RED} value for use in the VF calculation per the UVDGM-2006 requirement. Table 4-13 shows the data for the replicates at each set point. The highest RED bias at each set point is used in the validation factor calculations shown later in Section 4.8.4.

Table 4-13. RED Bias Factor for Each Set Point for *Giardia*

Sample Number	Test Run	UVT (%)	Sensitivity mJ/cm ² per Log I			B _{RED} 4.0-log <i>Giardia</i>	B _{RED} 3.5-log <i>Giardia</i>	B _{RED} 3.0-log <i>Giardia</i>
			RED	Log I	Sensitivity			
2-1	2	90.6	58.69	2.45	23.91	1.61	1.79	1.85
2-2	2	90.6	62.90	2.61	24.13	1.66	1.83	1.89
2-3	2	90.6	62.40	2.59	24.10	1.66	1.83	1.89
2-4	2	90.6	65.01	2.68	24.23	1.66	1.83	1.89
2-5	2	90.6	62.96	2.61	24.13	1.66	1.83	1.89
3-1	3	90.6	54.07	2.28	23.68	1.61	1.79	1.85
3-2	3	90.6	51.33	2.18	23.53	1.61	1.79	1.85
3-3	3	90.6	53.76	2.27	23.66	1.61	1.79	1.85
3-4	3	90.6	56.64	2.38	23.81	1.61	1.79	1.85
3-5	3	90.6	58.25	2.44	23.89	1.61	1.79	1.85
4-1	4	93.9	58.96	2.46	23.93	1.61	1.79	1.85
4-2	4	93.9	56.41	2.37	23.80	1.61	1.79	1.85
4-3	4	93.9	NA	NA	NA	NA	NA	NA
4-4	4	93.9	56.60	2.38	23.81	1.61	1.79	1.85
4-5	4	93.9	53.05	2.25	23.62	1.61	1.79	1.85
5-1	5	93.9	63.26	2.62	24.15	1.66	1.83	1.89
5-2	5	93.9	62.93	2.61	24.13	1.66	1.83	1.89
5-3	5	93.9	50.80	2.16	23.50	1.66	1.83	1.89
5-4	5	93.9	50.66	2.16	23.50	1.66	1.83	1.89
5-5	5	93.9	56.41	2.37	23.80	1.66	1.83	1.89
6-1	6	96.5	66.59	2.74	24.31	1.38	1.43	1.44
6-2	6	96.5	57.29	2.40	23.84	1.36	1.42	1.42
6-3	6	96.5	61.87	2.57	24.08	1.38	1.43	1.44
6-4	6	96.5	54.30	2.29	23.69	1.36	1.42	1.42
6-5	6	96.5	59.21	2.47	23.94	1.36	1.42	1.42
7-1	7	96.4	54.04	2.28	23.67	1.36	1.42	1.42
7-2	7	96.4	64.83	2.68	24.22	1.38	1.43	1.44
7-3	7	96.4	50.47	2.15	23.49	1.36	1.42	1.42
7-4	7	96.4	47.56	2.04	23.33	1.36	1.42	1.42
7-5	7	96.4	51.27	2.18	23.53	1.36	1.42	1.42
10-1	10	98.1	65.81	3.23	20.40	1.17	1.2	1.21
10-1	10	98.1	61.06	3.04	20.07	1.17	1.2	1.21
10-1	10	98.1	61.21	3.05	20.08	1.17	1.2	1.21
10-1	10	98.1	57.01	2.88	19.78	1.16	1.19	1.2
10-1	10	98.1	55.37	2.82	19.67	1.16	1.19	1.2
11-1	11	98.1	65.59	3.22	20.38	1.17	1.2	1.21
11-2	11	98.1	70.28	3.40	20.69	1.17	1.2	1.21
11-3	11	98.1	61.22	3.05	20.08	1.17	1.2	1.21
11-4	11	98.1	60.39	3.02	20.02	1.17	1.2	1.21
11-5	11	98.1	52.81	2.71	19.48	1.41	1.19	1.2
12-1	12	98.2	40.50	2.18	18.55	1.16	1.19	1.20
12-2	12	98.2	43.09	2.30	18.75	1.16	1.19	1.20
12-3	12	98.2	50.85	2.63	19.34	1.16	1.19	1.20
12-4	12	98.2	44.46	2.36	18.86	1.16	1.19	1.20
12-5	12	98.2	46.77	2.46	19.03	1.16	1.19	1.20

Sample Number	Test Run	UVT (%)	Sensitivity mJ/cm ² per Log I			B _{RED} 4.0-log <i>Giardia</i>	B _{RED} 3.5-log <i>Giardia</i>	B _{RED} 3.0-log <i>Giardia</i>
			RED	Log I	Sensitivity			
13-1	13	98.2	53.28	2.73	19.51	1.16	1.19	1.20
13-2	13	98.2	51.10	2.64	19.36	1.16	1.19	1.20
13-3	13	98.2	51.82	2.67	19.41	1.16	1.19	1.20
13-4	13	98.2	57.78	2.91	19.84	1.16	1.19	1.20
13-5	13	98.2	49.65	2.58	19.25	1.16	1.19	1.20
14-1	14	98.1	48.43	2.53	19.16	1.16	1.19	1.20
14-2	14	98.1	41.20	2.21	18.60	1.16	1.19	1.20
14-3	14	98.1	48.70	2.54	19.18	1.16	1.19	1.20
14-4	14	98.1	45.19	2.39	18.91	1.16	1.19	1.20
14-5	14	98.1	44.12	2.34	18.83	1.16	1.19	1.20
15-1	15	98.2	46.35	2.44	19.00	1.16	1.19	1.20
15-2	15	98.2	42.82	2.29	18.73	1.16	1.19	1.20
15-3	15	98.2	52.97	2.72	19.49	1.16	1.19	1.20
15-4	15	98.2	45.16	2.39	18.91	1.16	1.19	1.20
15-5	15	98.2	51.57	2.66	19.39	1.16	1.19	1.20
Maximum B_{RED}		Set Point 151 gpm - 7.9 mW/cm ²				1.66	1.83	1.89
		Set Point 251 gpm - 10.4 mW/cm ²				1.66	1.83	1.89
		Set Point 434 gpm - 13.2 mW/cm ²				1.38	1.43	1.44

NA - sample not analyzed so RED and bias not determined

4.8.3 Uncertainty of Validation

As described in Section 4.7.3, the uncertainty of validation (U_{Val}) addresses many sources of experimental uncertainty. The same approach to uncertainty calculations described in Section 4.7.3 apply to calculating the VF for *Giardia* and in fact the uncertainty values are the same. Please refer to Section 4.7.3 for the equations and discussion of the various uncertainty factors that are used for determining U_{Val} at each set point.

The U_{SP} results based on the RED_{meas} and standard deviation are shown in Table 4-9. The highest U_{SP} for each set point is 29.08% (151 gpm set point), 30.23% (251 gpm set point), and 34.54% (434 gpm set point).

The uncertainty of the validation is then calculated using the highest U_{DR} (32.58%) from the dose response data and the highest U_{SP} for each test condition using the equation:

$$U_{Val} = (U_{SP}^2 + U_{DR}^2)^{1/2}$$

Table 4-14 shows the U_{Val} values used for determining the uncertainty of the validation at each set point.

Table 4-14. Uncertainty of the Validation (U_{Val}) and B_{RED} Values for *Giardia*

Set Point	Max U_{DR} (%)	Max U_{SP} (%)	U_{Val} %	Max B_{RED}		
				4.0-log	3.5-log	3.0-log
151 gpm - 7.9 mW/cm ²	32.58	29.08	43.67	1.66	1.83	1.89
251 gpm - 10.4 mW/cm ²	32.58	30.23	44.44	1.66	1.83	1.89
434 gpm - 13.2 mW/cm ²	32.58	34.54	47.48	1.38	1.43	1.44

4.8.4 Validated Dose for *Giardia*

After establishing the U_{Val} and the RED bias as described above, the VF is calculated using the equation:

$$VF = B_{RED} \times [1 + (U_{Val} / 100)]$$

Where:

VF = Validation Factor;

B_{RED} = RED bias factor for *Giardia* (see Table 4-13); and

U_{Val} = Uncertainty of validation expressed as a percentage.

The validated dose is then calculated as follows:

$$\text{Validated dose (RED}_{Val}) = \text{RED}_{meas} / VF$$

Table 4-15 shows the calculated VF for various *Giardia* log inactivation levels (3.0, 3.5, and 4.0 log inactivation).

Table 4-15 shows the RED_{Val} for *Giardia* for each test run using the validation factors for the various *Giardia* log inactivation levels. Table 4-15 also shows the Validated Dose for each set point and a comparison to the dose required for various levels of inactivation of *Giardia*. As can be seen, the tests for the first and third set points (151 and 434 gpm) show a validated dose for *Giardia* that would achieve a minimum of 4.0 log inactivation. The second set point (middle point) at 251 gpm showed a minimum of 3.5 log inactivation.

The NeoTech D438TM achieved a minimum of 3.5 log inactivation for the low power runs at 251 gpm at 10.4 mW/cm² and a 4.0 log inactivation for all of the test runs at the set points at 151 gpm - 7.9 mW/cm² and 434 gpm - 13.2 mW/cm².

Table 4-15 Validation Factors and Validated Dose (RED_{Val}) for *Giardia*

Condition	Run #	Flow Rate (gpm)	Intensity (mW/cm ²)	Validation Factor			RED _{meas} (mJ/cm ²)	RED _{Val}		
				4.0-log	3.5-log	3.0-log		4.0-log (mJ/cm ²)	3.5-log (mJ/cm ²)	3.0-log (mJ/cm ²)
								22 ⁽¹⁾	15 ⁽¹⁾	12 ⁽¹⁾
Lowered UVT	2	151.1	7.9	2.38	2.63	2.72	62.39	26.2	23.7	23.0
Lowered UVT Dup	3	151.3	7.9	2.38	2.63	2.72	54.81	23.0	20.8	20.2
Lowered Power	10	154.8	7.9	2.38	2.63	2.72	60.09	25.2	22.9	22.1
Lowered Power Dup	11	154.1	7.9	2.38	2.63	2.72	62.06	26.0	23.6	22.9
Lowered UVT	4	251.4	10.0	2.40	2.64	2.73	56.26	23.5	21.3	20.6
Lowered UVT Dup	5	250.8	10.0	2.40	2.64	2.73	56.81	23.7	21.5	20.8
Lowered Power	12	251.8	10.4	2.40	2.64	2.73	45.13	18.8	17.1	16.5
Lowered Power Dup	13	251.6	10.4	2.40	2.64	2.73	52.73	22.0	19.9	19.3
Lowered UVT	6	436.0	13.0	1.99	2.07	2.08	59.85	30.0	29.0	28.8
Lowered UVT Dup	7	435.5	13.0	1.99	2.07	2.08	53.63	26.9	26.0	25.8
Lowered Power	14	434.2	13.1	1.99	2.07	2.08	45.53	22.8	22.0	21.9
Lowered Power Dup	15	435.8	13.2	1.99	2.07	2.08	47.77	24.0	23.1	23.0

(1) Required dose for log inactivation validation per the UVDGM-2006 Appendix G.

4.9 Validated Dose (RED_{val}) for MS2 as the Target Organism

Some regulatory agencies, such as the NYDOH, have established a standard for spray parks and other applications based on a validated dose (RED_{val}) of 40 mJ/cm² based on MS2. The calculation of the validation factor for a validated dose based on MS2 is performed using B_{RED} set equal to 1.0. For MS2 validated dose calculations, B_{RED} is set equal to 1.0 because the pathogen selected, namely MS2, is the same as the test organism, so there is no bias correction. Therefore, the validation factor will not vary by the log inactivation level.

The U_{val} is calculated in the same manner as described in Section 4.7.3.

The VF for evaluating validated dose (RED_{val}) based on MS2 is calculated using the same formula as for other pathogens as follows:

$$VF = B_{RED} \times [1 + (U_{val} / 100)]$$

Where:

VF = Validation Factor;

B_{RED} = RED bias factor (set equal 1.0); and

U_{val} = Uncertainty of validation expressed as a percentage.

The validated dose is then calculated as follows:

$$\text{Validated dose } (RED_{val}) = RED_{observed} / VF$$

Table 4-16 shows the RED_{val} based on MS2 for each test run.

Using the VF calculated for each set point, the RED_{val} based on MS2 was calculated for each test run. Only some of the test runs achieved a 40 mJ/cm² validated dose based on MS2. The lowest flow rate condition (151 gpm) showed that three of the four test runs achieved a validated dose of 40 mJ/cm². One run was at 38 mJ/cm², just below the RED_{val} target of 40 mJ/cm² based on MS2. A main influence on the validated dose for the lower flow tests is the U_{DR} being just above the 30% level at 32.58%. If the U_{DR} had been less than or equal to 30%, the validation factor would be significantly lower. In that case, all of the 151 gpm set point test runs would be > 40 mJ/cm² and three of the four 251 gm set point test runs would be above 40 mJ/cm².

Table 4-16. Validation Factors and Validated Dose (RED_{val}) based on MS2

Condition	Run	Flow Rate (gpm)	Intensity (mW/cm²)	Validation Factor (1)	RED_{meas} (mJ/cm²)	RED_{val} (mJ/cm²)
Lowered UVT	2	151	7.9	1.44	62.39	43.4
Lowered UVT Dup	3	151	7.9	1.44	54.81	38.2
Lowered Power	10	155	7.9	1.44	60.09	41.8
Lowered Power Dup	11	154	7.9	1.44	62.06	43.2
Lowered UVT	4	251	10.0	1.44	56.26	38.9
Lowered UVT Dup	5	251	10.0	1.44	56.81	39.3
Lowered Power	12	252	10.4	1.44	45.13	31.2
Lowered Power Dup	13	252	10.4	1.44	52.73	36.5
Lowered UVT	6	436	13.0	1.47	59.85	40.6
Lowered UVT Dup	7	436	13.0	1.47	53.63	36.4
Lowered Power	14	434	13.1	1.47	45.53	30.9
Lowered Power Dup	15	436	13.2	1.47	47.77	32.4

(1) B_{RED} equal to 1.0, therefore all log inactivation levels have the same validation factor.

4.10 Water Quality Data

Samples were collected for general water quality characterization. Influent and effluent samples were collected during each flow test run and analyzed for temperature, pH, total chlorine, and free chlorine. An influent sample was collected from each flow test run and analyzed for turbidity, iron, and manganese.

An influent and effluent sample from each test run was also collected and analyzed for total coliform, *E. coli*, and HPC.

The general chemistry and microbiological results are presented in Tables 4-17 through 4-20.

Table 4-17. Temperature and pH Results

Test	Run #	Temperature (°F)		pH (S.U.)	
		Influent	Effluent	Influent	Effluent
Blank	1	72.3	72.2	7.39	7.41
Low UVT (91%) Full power	2	72.4	72.3	7.42	7.38
Low UVT (91%) Full power Dup	3	72.5	72.3	7.41	7.39
Low UVT (94%) Full Power	4	72.5	72.5	7.52	7.52
Low UVT (94%) Full Power Dup	5	72.3	72.4	7.57	7.55
Low UVT (97%) Full power	6	73.3	73.2	8.28	8.28
Low UVT (97%) Full power Dup	7	72.6	72.6	8.43	7.47
Blank	8	72.0	72.0	8.37	8.35
Reactor Control	9	71.8	71.8	8.36	8.37
Reduced Power - High UVT	10	71.4	71.4	8.36	8.37
Reduced Power - High UVT Dup	11	71.3	71.4	8.35	8.37
Reduced Power - High UVT	12	72.1	72.1	8.48	8.48
Reduced Power - High UVT Dup	13	72.0	72.0	8.51	8.53
Reduced Power - High UVT	14	72.2	72.0	8.60	8.58
Reduced Power - High UVT Dup	15	72.4	72.4	8.64	7.64

Table 4-18. Total Chlorine, Free Chlorine and Turbidity Results

Test	Run #	Total Chlorine (mg/L)	Free Chlorine (mg/L)	Turbidity (NTU)
		Influent	Influent	Influent
Blank	1	<0.02	<0.02	0.45
Low UVT (91%) Full power	2	<0.02	<0.02	0.41
Low UVT (91%) Full power Dup	3	<0.02	<0.02	0.44
Low UVT (94%) Full Power	4	<0.02	<0.02	0.36
Low UVT (94%) Full Power Dup	5	<0.02	<0.02	0.34
Low UVT (97%) Full power	6	<0.02	<0.02	0.23
Low UVT (97%) Full power Dup	7	<0.02	<0.02	0.23
Blank	8	<0.02	<0.02	0.22
Reactor Control	9	<0.02	<0.02	0.28
Reduced Power - High UVT	10	<0.03	<0.02	0.18
Reduced Power - High UVT Dup	11	<0.02	<0.02	0.19
Reduced Power - High UVT	12	<0.02	<0.02	0.17
Reduced Power - High UVT Dup	13	<0.02	<0.02	0.21
Reduced Power - High UVT	14	<0.02	<0.02	0.14
Reduced Power - High UVT Dup	15	<0.02	<0.02	0.20

Table 4-19. Iron and Manganese Results

Test	Run #	Iron (mg/L)	Manganese (mg/L)	UVT ⁽¹⁾ (%)	
		Influent	Influent	Influent	Effluent
Blank	1	0.03	<0.001	91	91
Low UVT (91%) Full power	2	0.05	<0.001	91	90
Low UVT (91%) Full power Dup	3	0.05	<0.001	91	90
Low UVT (94%) Full Power	4	<0.02	<0.001	93	93
Low UVT (94%) Full Power Dup	5	0.02	<0.001	93	92
Low UVT (97%) Full power	6	<0.02	<0.001	95	95
Low UVT (97%) Full power Dup	7	0.72	0.004	95	95
Blank	8	<0.02	<0.001	96	96
Reactor Control	9	<0.02	<0.001	96	96
Reduced Power - High UVT	10	<0.02	<0.001	96	96
Reduced Power - High UVT Dup	11	<0.02	<0.001	96	96
Reduced Power - High UVT	12	<0.02	<0.001	96	96
Reduced Power - High UVT Dup	13	<0.02	0.001	96	96
Reduced Power - High UVT	14	<0.02	<0.001	96	96
Reduced Power - High UVT Dup	15	<0.02	<0.001	96	96

(1)UVT on grab samples, measured in laboratory after tests; In- line UVT meter used for flow test results

Table 4-20. HPC, Total Coliform and *E. coli* Results

Test	Run #	Total Coliform (MPN/100mL)		<i>E. coli</i> (MPN/100mL)		HPC (CFU/mL)	
		Influent	Effluent	Influent	Effluent	Influent	Effluent
Blank	1	2	<1	<1	<1	4.76E+03	3.95E+01
Low UVT (91%) Full power	2	8.66E+02	<1	<1	<1	7.17E+03	2.35E+00
Low UVT (91%) Full power Dup	3	5.94E+01	<1	<1	<1	3.99E+03	2.10E+01
Low UVT (94%) Full Power	4	3.17E+01	<1	<1	<1	2.80E+03	1.33E+02
Low UVT (94%) Full Power Dup	5	6.30E+00	<1	<1	<1	3.11E+03	2.60E+01
Low UVT (97%) Full power	6	1.60E+01	<1	<1	<1	1.34E+03	1.28E+02
Low UVT (97%) Full power Dup	7	4.10E+01	<1	<1	<1	4.65E+02	4.15E+01
Blank	8	<1	<1	<1	<1	1.61E+03	1.15E+02
Reactor Control	9	1.05E+03	1.05E+03	<1	<1	3.62E+03	4.20E+03
Reduced Power - High UVT	10	1.57E+02	7.76E+01	<1	<1	1.75E+03	4.80E+01
Reduced Power - High UVT Dup	11	7.76E+01	<1	<1	<1	2.82E+03	1.85E+01
Reduced Power - High UVT	12	1.34E+01	<1	<1	<1	9.45E+02	6.10E+01
Reduced Power - High UVT Dup	13	5.20E+00	<1	<1	<1	3.70E+02	1.50E+01
Reduced Power - High UVT	14	4.10E+00	<1	<1	<1	4.65E+02	4.70E+01
Reduced Power - High UVT Dup	15	1.46E+01	<1	<1	<1	7.20E+02	4.65E+01

4.11 Power Measurement

A power monitoring platform was connected to the unit. This monitoring platform provided continuous readout of the volts, amperage, and watts being used by the unit under each test condition. Volts, amperes, and wattage were recorded during each flow test. The power factor was also recorded. Table 4-21 presents the power measurements taken during the flow tests.

Table 4-21. Power Measurement Results

Test	Run #	Volts (volts)	Amperage (amps)	Watts (watts)	Power Factor
Blank	1	117	3.0	340	0.97
Low UVT (91%) Full power	2	117	3.0	340	0.96
Low UVT (91%) Full power Dup	3	116	3.0	340	0.96
Low UVT (94%) Full Power	4	117	3.0	340	0.97
Low UVT (94%) Full Power Dup	5	117	3.0	340	0.96
Low UVT (97%) Full power	6	117	3.0	340	0.96
Low UVT (97%) Full power Dup	7	117	3.0	340	0.97
Blank	8	118	2.0	230	0.99
Reactor Control	9	119	0.0	0.0	--
Reduced Power - High UVT	10	117	2.0	230	0.98
Reduced Power - High UVT Dup	11	117	2.0	230	0.98
Reduced Power - High UVT	12	116	3.0	340	0.97
Reduced Power - High UVT Dup	13	NR	NR	NR	NR
Reduced Power - High UVT	14	116	3.5	390	0.96
Reduced Power - High UVT Dup	15	116	3.5	390	0.96

NR-not reported because the data were not recorded on log sheet

4.12 Headloss

Headloss was measured over the flow range of 100 to 450 gpm. Pressure at the inlet and outlet of the reactor was measured at several flow rates as shown in Table 4-22.

Table 4-22. Headloss Measurement Results

Flow Rate	Inlet (psi)	Outlet (psi)	Headloss (psi)
100	1.59	1.20	0.39
150	1.92	1.29	0.63
250	2.87	1.52	1.35
350	4.22	1.88	2.34
450	6.16	2.35	3.81

Chapter 5

Quality Assurance/Quality Control

5.1 Introduction

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

Because this 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.

5.2 Test Procedure QA/QC

NSF testing laboratory staff conducted the tests by following a USEPA-approved TQAP created specifically for this verification. NSF QA Department staff performed an 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 and Microbiology Laboratories were labeled with unique identification numbers. All samples were analyzed within allowable holding times.

5.4 Chemistry Laboratory 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 method has an NSF standard operating procedure.

The bench top UV spectrophotometer was calibrated with Holmium Oxide with each batch of samples analyzed. The peaks at 241.1, 287.6 and 293.5 nm all passed QC criteria for peak intensity and peak nm location. Dichromate standards were also run with each batch of samples and found to be within 102% of the true value.

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.

5.5.2 Negative Controls

For each sample batch processed, an unused membrane filter and a blank with 100 mL of buffered, sterilized dilution water was 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 Collimated Beam Apparatus and QA/QC

The petri dish factor was determined for the collimated beam apparatus prior to the start of the test program. Radiometers were calibrated and checked in accordance with operating procedure and UVDGM-2006 requirements. These procedures and data were reviewed as part of the NSF QA department review of the microbiological laboratory data.

NSF received reviewer comments about the collimated beam data in the Draft EPA ETV NSF UV reports in November of 2011. They identified two issues related to collimated beam data for NSF to investigate. The two issues were a high degree of uncertainty with replicates in the collimated beam data and the data trending at or below the lower 95% confidence interval for MS2 UV sensitivity. The initial investigation revealed no systematic error. However, further investigation revealed a miscommunication between the company that calibrated NSF's radiometers and NSF. It was learned that the radiometers were calibrated at one of two settings and that NSF used the setting that the radiometer was not calibrated at. Hence the MS2 had received about 25% more UV dose than estimated by the collimated beam data. Therefore, the calculated RED_{meas} at each set point was lower than the actual dose delivered by the unit. NSF determined that the best action was to retest all previous tested units. That testing was done in the summer of 2012 and is reported in this report. All previous data is not reported herein as it was deemed to be biased.

The factors used in the collimated test shown below were evaluated against the protocol requirements and found to meet the QC objectives. The length (distance from the lamp centerline to the suspension) and the depth of suspension were fixed parameters. These measurements were made multiple times at the "fixed mark" on the collimated beam apparatus to estimate the precision of the measurements. The time was checked based on a stop watch with minimal uncertainty. The petri dish factor was measured four times prior to the start of the test. Absorbance uncertainty is based on spectrophotometer precision, as is the related reflectance factor. The average intensity is measured for every collimated beam test, as it is required that intensity be measured before and after each test.

To control for error in the UV dose measurement, the uncertainties of the terms in the UV dose calculation met the following criteria:

	Estimated	Required
Depth of suspension (d)	<5%	≤10%
Average incident irradiance (E_s)	2.5%	≤8%
Petri Factor (P_f)	2.1%	≤5%
$L/(d + L)$	0.7%	≤1%
Time (t)	1.6%	≤5 %
$(1 - 10^{-ad})/ad$	1.2%	≤5%

The collimated beam test procedures and key test parameters (radiometer intensity, petri dish factor, exposure time, suspension depth, distance from lamp to suspension surface, and absorbance/reflectance) were thoroughly reviewed for all days of testing.

The collimated beam data, as presented in Tables 4-3 and 4-4 show that on Day 2 of testing with the high UVT water, there was one outlier in replicate 2 for the collimated beam results. The outlier, as determined by the Grubb statistics presented in Table 4-3 was eliminated from the dose response curve and equation. Both sets of data, with and without the outlier are presented in Tables 4-3 and 4-4. As can be seen there was very little difference in the dose response relationship between the data set with the outlier included and with it removed.

The dose response curve for the collimated beam data were within the QC boundaries set in the GP-2011 and UVDGM-2006. The boundaries are shown on Figures 4-3 and 4-4 along with all of the collimated beam dose response data. The low UVT water collimated beam data did have two data points that were on the upper boundary line and the curve was on the high side of the midpoint. However, the dose response curve was within the established QC boundaries.

Stability tests for MS2 are normally performed to show that the phage does not change during holding times when samples are shipped from the test site to the laboratory and/or held in the laboratory prior to analysis. For these tests, the test rig was located in the same building as the microbiology laboratory. Samples were delivered to the laboratory after each test run and the laboratory ran the samples within 4 to 6 hours of sample collection. Therefore, stability samples were not necessarily needed for these test runs, as the holding time was very short. However, stability tests were run for the times 0, 4, 8, 24 hours for informational purposes. Table 5-1 shows the stability test results.

Trip blanks are also normally performed to show that the stock phage solution does not change during shipment to and from the test site. The phage stock solution was delivered from the microbiology before each test run and the samples were returned to the laboratory after each test run. Therefore trip blanks were not required for these tests, as all stock solution and test samples were received from and delivered to the microbiology laboratory before/after each test run. No shipping or long holding times were required. However, trip blanks were performed once each day to demonstrate that no significant change was occurring during transport and handling of the samples. Table 5-2 shows the trip blank results.

Table 5-1. Stability Results

	Low UVT Water				High UVT Water		
	MS2				MS2		
	PFU/mL	Average	log ₁₀		PFU/mL	Average	log ₁₀
Influent 0 Hour	1.07E+05	1.17E+05	5.07	Influent 0 Hour	1.20E+05	1.18E+05	5.07
	1.20E+05				1.21E+05		
	1.24E+05				1.14E+05		
Influent 4 Hour	4.80E+04	4.80E+04	4.68	Influent 4 Hour	6.30E+04	7.77E+04	4.89
	4.10E+04				8.30E+04		
	5.50E+04				8.70E+04		
Influent 8 Hour	5.30E+05	4.80E+05	5.68	Influent 8 Hour	5.70E+05	5.17E+05	5.71
	4.60E+05				5.40E+05		
	4.50E+05				4.40E+05		
Influent 24 Hour	4.60E+05	4.70E+05	5.67	Influent 24 Hour	7.10E+05	6.43E+05	5.81
	5.10E+05				6.80E+05		
	4.40E+05				5.40E+05		
Effluent 0 Hour	9.40E+02	9.30E+02	2.97	Effluent 0 Hour	1.06E+03	9.37E+02	2.97
	8.90E+02				8.80E+02		
	9.60E+02				8.70E+02		
Effluent 4 Hour	1.83E+03	1.09E+03	3.04	Effluent 4 Hour	4.20E+02	4.83E+02	2.68
	8.50E+02				5.10E+02		
	6.00E+02				5.20E+02		
Effluent 8 Hour	4.90E+03	3.48E+03	3.54	Effluent 8 Hour	2.56E+03	2.28E+03	3.36
	2.43E+03				2.04E+03		
	3.10E+03				2.23E+03		
Effluent 24 Hour	5.20E+03	4.37E+03	3.64	Effluent 24 Hour	6.60E+03	5.73E+03	3.76
	3.70E+03				6.50E+03		
	4.20E+03				4.10E+03		

Table 5-2. Trip Blank Results

	Day 1 (08-02-12)			Day 2 (08-03-12)	
	MS2			MS2	
	PFU/mL	Log ₁₀		PFU/mL	Log ₁₀
Held in Micro Lab	6.07E+08	8.78		7.15E+08	8.85
Travel to Test Setup	5.40E+08	8.73		7.40E+08	8.87
Difference		0.05			0.02

5.6 Engineering Lab - Test Rig QA/QC

The flow meter for the test rig is part of the NSF tank, pump, and flow control system used for UV testing and other tests in the engineering laboratory. The flow meter is calibrated by the NSF

QA staff at least annually. Calibration is performed by measuring the draw down volume from the calibrated feed tank over time. The tank was calibrated by filling with measured volumes of water and the corresponding depth measured. In addition to the annual calibration, the flow meter was calibrated after the test runs were completed. Calibration was performed at 150, 250, 350, and 450 gpm covering the range of flow rates tested. The calibration of the flow meter showed accuracy of $\leq 1.66\%$ (average of 1.45% over the flow range) and easily met the requirement of $\pm 5\%$. Table 5-3 presents the flow meter calibration data.

Table 5-3. Flow Meter Calibration Data

Flow Meter Reading (gpm)	Actual Calculated Flow Rate based on tank level change and time (gpm)	Deviation (%)
453.5	460.1	1.44
346.0	351.8	1.66
254.0	256.0	0.78
150.3	153.2	1.91
Average		1.45

Reactor control and reactor blanks were performed as part of the validation. One reactor control, with MS2 coliphage injection, and the lamps off, was performed to demonstrate that the MS2 concentration was not changing as the seeded water passed through the reactor. Reactor blanks were collected for each day of testing to demonstrate that the system was not accumulating or being contaminated with MS2 at levels that would interfere with the test.

Table 5-4 presents the results of the reactor control and reactor blanks. The reactor control had an average influent concentration of 5.74 \log_{10} and an average effluent concentration of 5.76 \log_{10} showing an increase of 0.02 \log_{10} through the system with lamps off. This meets the criteria of less than a 0.2 \log_{10} change through the unit with lamps turned off.

During the first day of testing, the reactor blanks showed no detectable MS2 (<1 PFU/ml) present in either the influent or effluent samples. On Day 2, the influent showed some MS2 present at 20 to 23 PFU/mL (1.30 to 1.36 \log_{10}) which is above the objective concentration of less than 0.2 \log_{10} . The effluent samples were clean (<1 PFU/mL). The presence of some MS2 in the influent did not directly impact the data analysis, as the amount of MS2 was small compared the influent concentration based on an injection rate of $> 5.0 \log_{10}$.

The results for the blank samples for HPC, total coliform, and *E. coli* were presented in Table 4-20.

Table 5-4. Reactor Control and Reactor Blank MS2 Results

Test Condition	Test Day	Test Run	UVT (%)	Flow (gpm)	Intensity (mW/cm ²)	Influent (PFU/mL)			Effluent (PFU/mL)				
						Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3		
Reactor Blank	1	1	91	150	8.0	<1	<1	<1		<1	<1	<1	
Reactor Blank	2	8	98	150	7.9	23	22	20		<1	<1	<1	
Reactor Control	2	9	98	154	0.0	6.50E+05	5.50E+05	5.53E+05		8.40E+05	4.67E+05	5.47E+05	
Test Condition	Test Day	Test Run	UVT (%)	Flow (gpm)	Intensity (mW/cm ²)	Influent log ₁₀			Ave	Effluent log ₁₀			Ave
						Rep 1	Rep 2	Rep 3		Rep 1	Rep 2	Rep 3	
Reactor Blank	1	1	91	150	8.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Reactor Blank	2	8	98	150	7.9	1.36	1.34	1.30	1.33	0.00	0.00	0.00	0.00
Reactor Control	2	9	98	154	0.0	5.81	5.74	5.74	5.76	5.92	5.67	5.74	5.78

5.7 Documentation

All laboratory activities were documented using specially prepared laboratory bench sheets and NSF laboratory reports. Data from laboratory reports were entered into Microsoft™ Excel® spreadsheets. These spreadsheets were used to calculate the means and log₁₀ inactivations. One hundred percent of the data entered into the spreadsheets was checked by a reviewer to confirm all data and calculations were correct.

5.8 Data Review

NSF QA/QC staff reviewed the raw data records for compliance with QA/QC requirements. As required in the ETV Quality Management Plan, NSF ETV staff checked at least 10% of the data in the NSF laboratory reports against the lab bench sheets.

5.9 Data Quality Indicators

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

5.9.1 Representativeness

Representativeness is a qualitative term that expresses “the degree to which data accurately and precisely represent a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition.” 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.

5.9.2 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, where applicable, and certified standards during calibration of an instrument.

The following equation was used to calculate percent recovery:

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

where:

$$\begin{aligned} X_{\text{known}} &= \text{known concentration of the measured parameter} \\ X_{\text{measured}} &= \text{measured concentration of parameter} \end{aligned}$$

Accuracy of the bench top chlorine, pH, and turbidity meters was checked daily during the calibration procedures using certified check standards. The in-line UVT monitor was calibrated daily with both a purchased UVT standard and with DI water at 99.9% UVT, before the flow tests.

The NSF Laboratory Quality Assurance Manual establishes the frequency of spike sample analyses at 10% of the samples analyzed for chemical analyses. Laboratory control samples are also run at a frequency of 10%. The recovery limits specified for the parameters in this verification, excluding microbiological analyses, were 70-130% for laboratory-fortified (spiked) samples and 85-115% for laboratory control samples. The NSF QA department reviewed the laboratory records and found that all recoveries were within the prescribed QC requirements. Calibration requirements were also achieved for all analyses.

5.9.3 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 iron and manganese measurement. At least one out of every ten samples for pH, total chlorine, free chlorine, temperature, and turbidity was analyzed in duplicate as part of the daily calibration process. Precision of duplicate analyses was measured by use of the following equation to calculate Relative Percent Deviation (RPD):

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

where:

S_1 = sample analysis result; and

S_2 = sample duplicate analysis result.

Acceptable analytical precision for the verification test was set at an RPD of 30%. Field duplicates were collected at a frequency of one out of every 10 samples for each parameter, to incorporate both sampling and analytical variation to measure overall precision against this objective. In addition, the NSF Laboratory also conducted laboratory duplicate measurements at 10% frequency of samples analyzed. The laboratory precision for the methods selected was tighter than the 30% overall requirement, generally set at 20% based on the standard NSF Chemistry Laboratory method performance.

All RPD were within NSF's established allowable limits for each parameter.

5.9.4 Completeness

Completeness is the proportion of valid, acceptable data generated using each method as compared to the requirements of the TQAP 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, as presented in Table 5-5.

Table 5-5. 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.

One hundred percent completeness was achieved for all aspects of this validation except for the MS2 run samples, power measurements, and collimated beam analyses. Two samples (one influent and one effluent - test run 4) were not analyzed out of a total of 120 samples scheduled during the main test runs. The completeness is calculated as 98.3% complete for the MS2 test run samples. One power measurement was not recorded out of a total of 15 scheduled measurements for a completeness of 93.3%. There was one outlier result for replicate 2 from the collimated beam tests on the high UVT water. A total of 24 samples were scheduled and 23 used for the dose response relationship. Therefore, completeness was 95.8%. All planned testing activities were conducted as scheduled.

Chapter 6 References

1. Test/Quality Assurance Plan for The Ultraviolet Sciences Inc. Ultraviolet (UV) Water Purification System Model UVS438s-500 Reactor, August 2010.
2. Generic Protocol for Development of Test/Quality Assurance Plans for Validation of Ultraviolet (UV) Reactors, NSF International, 7/2010.
3. Generic Protocol for Development of Test / Quality Assurance Plans for Validation of Ultraviolet (UV) Reactors, 10/01/EPADWCTR, August 2011.
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5. German Association for Gas and Water (DVGW) Technical Standard Work Sheet W 294-1,2,3 (June 2006).
6. Austrian Standards, ÖNORM M5873-1, Plants for the disinfection of water using ultraviolet radiation, Requirements and testing, Low pressure mercury lamp plants (March, 2001).
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10. Water Report 113, Safe, Sufficient and Good Potable Water Offshore: A guideline to design and operation of offshore potable water systems. 2nd edition. By Eyvind Andersen and Bjørn E. Løfsgaard, Norwegian Institute of Public Health, November 2009.
11. Recommended Standards For Water Works, Policies for the Review and Approval of Plans and Specifications for Public Water Supplies, 2012 Edition, A Report of the Water Supply Committee of the Great Lakes--Upper Mississippi River Board of State and Provincial Public Health and Environmental Managers.

Attachment 1

NeoTech D438™ Technical Manual and Specifications

Contact Mr. Bruce Bartley at 734-769-5148 or bartley@nsf.org for a copy of this document.

This attachment is a large pdf file.

Attachment 2

Additional Sensor and Lamp Information

Contact Mr. Bruce Bartley at 734-769-5148 or bartley@nsf.org for a copy of this document.

This attachment is a pdf file.

Attachment 3

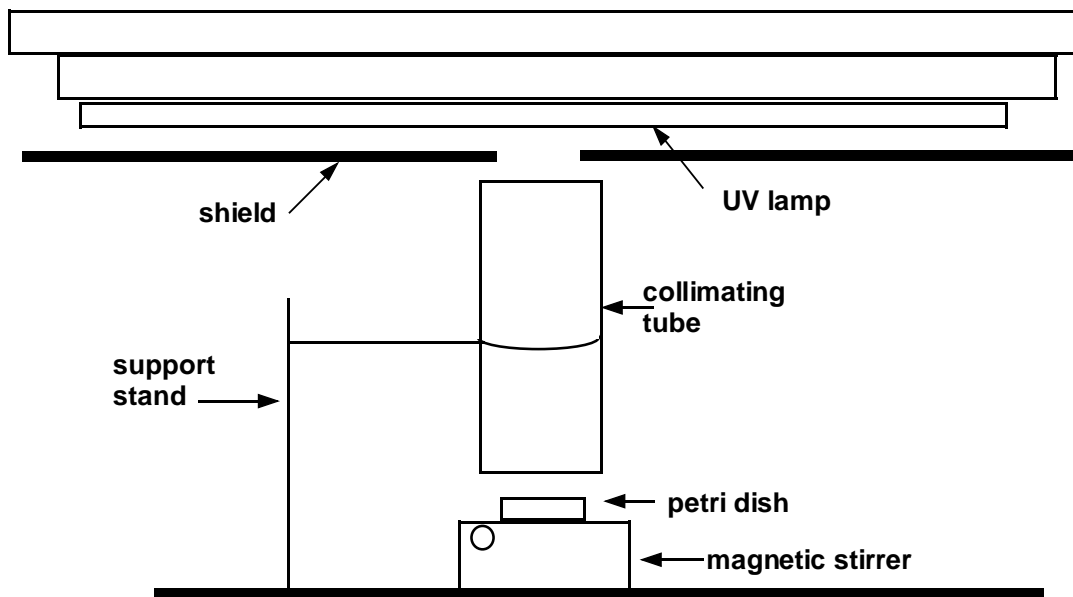
NSF Collimated Beam Apparatus

Description of the Collimated Beam Apparatus

(NSF Standard 55 7.2.1.2)

An apparatus shall be assembled in which a small stirred sample can be irradiated in a nearly collimated beam. A radiometer meeting specification in 7.2.1.2.1 can then be used to measure the incident irradiance (E_0).

A low-pressure mercury vapor UV lamp shall be wired to a ballast and a voltage regulator (figure 2). A solution contained in a small dish equal to or smaller in diameter than that of the collimated tube shall be used. The solution shall be 1 cm deep. E_0 shall be measured at the surface of the liquid by removing the dish and stirrer and placing the radiometer at the corresponding position from which the dish was removed. The UV irradiance at each point of the surface shall be within $\pm 5\%$ of the average irradiance across the solution surface.



NOTE 1 – The collimating tubes shall be a minimum of 53 cm (21 in) in length and the interior shall be painted flat black.

NOTE 2 – The support stand, if used, shall be adjustable to raise or lower the collimating tube to the surface of the petri dish.

NOTE 3 – The petri dish shall be set so the surface of the liquid is at the same level as the radiometer.

NOTE 4 – Measurement of the UV dose must be done at the same point at which the petri dish surface is exposed.

Figure 2 – Collimated beam apparatus

7.2.1.2.1 Radiometer specifications

A radiometer with the following specification shall be used:

- linearity: $\pm 0.5\%$;

- spectral response: visible-blind detector with narrow band-pass filter centered at 254 nm, full width at half maximum = 20 nm or less;
- spatial response: cosine response $\pm 5\%$;
- calibration: Radiometer calibration (including optics, transducer and electronics) shall be traceable to the National Institute of Standards and Technology (NIST) or another national standards laboratory. Calibration shall be performed annually or at the intervals specified by manufacturer, whichever is more frequent;

$$E_{ave} = 0.98 \left[\frac{E_0}{L} \left(\frac{(T)^L - 1}{\ln[T]} \right) \right]$$

- uncertainty: The calibration documentation provided with each radiometer (including optics, transducer, and electronics) shall include both calibration uncertainties (transfer uncertainty to customer) and the uncertainty associated with the calibration standard. The NIST (or other national laboratory) uncertainty is added the transfer uncertainty to customer to yield total uncertainty; and
- maximum total uncertainty: $\pm 9\%$ at 254 nm.

Method for Challenge Microorganism Preparation, Culturing the Challenge Organism and Measuring its Concentration

(NSF Standard 55 Annex A)

A.1 Summary

MS-2 Coliphage is used as the biological surrogate to determine the average UV dose output of UV water treatment systems. The methods that are used for suspension preparation, titration, and analysis of the challenge organisms for use in the sensitivity calibration and testing are presented in this annex.

A.2 Equipment

- autoclave;
- radiometer (International light IL-700);
- UV collimating beam apparatus and 254 nm photo detector;
- incubator, 35 ± 1 °C (95 ± 1 °F);
- refrigerator, 5 ± 3 °C (41 ± 3 °F);
- water bath 50 ± 1 °C (122 ± 1 °F);
- freezer;
- microwave;
- vortex mixer;
- UV-vis spectrophotometer;
- pH meter;
- hemocytometer;
- Colony Counter; and
- centrifuge.

A.3 Microorganisms

All organisms shall be obtained from ATCC.

- MS-2 Coliphage (ATCC # 15597-BI); and
- *Escherichia coli* host strain (ATCC # 15597).

A.4 Supplies

- Petri dishes, 20 x 60 mm and 15 x 100 mm: sterile;
- pipettes, 1 mL and 10 mL, sterile;
- sterile centrifuge tubes, 10 mL and 50 mL;
- sample bottles, 125 mL sterile screw cap;
- test tubes, 16 x 125 mm;
- sterile inoculating loop;
- sterile filtration apparatus;
- sterile 0.22 µm polycarbonate membrane filters;
- Whatman #1 filter;
- chlorine detection kit; and
- disposable sterile 250 mL polypropylene container.

A.5 Reagents

- Sterile buffered dilution water (SBDW). This shall be prepared according to the *Standard Methods for the Examination of Water and Wastewater* (dilution water: buffered water);
- Phosphate buffer saline (PBS). A stock solution shall be prepared by dissolving 80 g sodium chloride (NaCl), 2 g potassium dihydrogen phosphate (KH₂PO₄), 29 g hydrated disodium hydrogen phosphate (Na₂HPO₄·12H₂O), and 2 g potassium chloride (KCl) in water to a final volume of 1 L. A working solution shall be prepared from the stock solution by diluting 1 volume of the stock with 9 volumes of water. The pH shall be adjusted using a pH meter to 7.4 with 0.1 N HCl or 0.1 N NaOH before use;
- Ethylenediaminetetraacetic acid (EDTA), Sigma # ED2SS; and
- Lysozyme, Boehringer Mannheim, #1 243004. Store at 2 to 8 °C (35 to 46 °F).

A.6 Safety precautions and hazards

A.6.1 Steam sterilized samples and equipment shall be handled with protective gloves when being removed from the autoclave.

A.6.2 Cryogenic culture vials shall be handled with cryoprotective gloves.

A.6.3 Ultraviolet light shall be used to expose the organism during calibration. This light can result in skin cancer and retinal damage; hence personnel must be protected from exposure.

A.6.4 All microbiological samples and contaminated test supplies shall be steam sterilized to 121 ± 1 °C (250 ± 1 °F) at 15 psi for a minimum of 20 min prior to being discarded.

A.7 Growth medium

NOTE 1 – Common bacteriological media may be purchased from bacteriological medium manufacturers and prepared according to the manufacturer's instructions.

NOTE 2 – The quality of the growth media shall be monitored by examining growth promotion and sterility prior to use.

A.7.2 Formula to be used when MS-2 Coliphage is chosen for microbiological agent

A.7.2.1 TSB (Tryptic Soy Broth)

Ingredient	Amount
tryptone	1.7 g
soytone	0.3 g
dextrose	0.25 g
sodium chloride	0.5 g
dipotassium phosphate	0.25 g
DI water	100 mL
pH	7.3 ± 0.2

TSB shall be dissolved by boiling and adjusted to final pH. 8-mL aliquots shall be dispensed into 16 x 150 mm test tubes. TSB shall be autoclaved at 121 ± 1 °C (250 ± 1 °F) at 15 psi for 20 min. Cooled broth shall be stored at 5 ± 1 °C (41 ± 1 °F).

A.7.2.2 1.5% TSA (Tryptic Soy Agar)

Ingredient	Amount
tryptone	7.5 g
soytone	2.5 g
sodium chloride	2.5 g
bacto-agar	7.5 g
DI water	500 mL
pH	7.3 ± 0.2

TSA shall be dissolved by boiling, adjusted to final pH, and autoclaved at 121 ± 1 °C (250 ± 1 °F) at 15 psi for 20 min. Tempered media shall be poured into sterile petri dishes. Agar plates shall be stored at 5 ± 1 °C (41 ± 1 °F). Plates shall be allowed to come to room temperature before use.

A.7.2.3 Phage top agar 1% TSA (Tryptic Soy Agar)

Ingredient	Amount
tryptone	7.5 g
soytone	2.5 g
sodium chloride	2.5 g
agar	5.0 g
DI Water	500 mL
pH	7.3 ± 0.2

TSA shall be dissolved by boiling, adjusted to final pH, and autoclaved at 121 ± 1 °C (250 ± 1 °F) at 15 psi for 20 min. Agar shall be stored at 5 ± 3 °C (41 ± 1 °F). On the day of testing, the TSA shall be liquefied and placed in the 45 ± 1 °C (113 ± 1 °F) water bath. The MS-2 Coliphage top agar shall be maintained at 45 ± 1 °C (113 ± 1 °F) to prevent agar solidification.

A.8 Culture of challenge organisms

A.8.2 MS-2 Coliphage

A.8.2.1 Stock culture preparation of MS-2 Coliphage

NOTE – This section describes the propagation and harvesting methods for stock suspensions of MS-2 Coliphage for use as a challenge suspension for low flow (< 1 gpm) water treatment units. If units possessing a flow rate greater than 1 gpm are to be tested, the stock preparation procedure may have to be repeated multiple times to achieve the required volume of MS-2 Coliphage. This method should also be repeated when cryogenic stocks are low.

a) One day prior to preparation of MS-2 Coliphage stock, a cryogenically frozen *E. coli* host strain shall be thawed. One TSB tube shall be inoculated with 0.1 mL of the stock suspension. The stock suspension shall be incubated at 35 ± 1 °C (95 ± 1 °F) for 18 ± 2 h.

b) On the day of preparing MS-2 Coliphage stock, 1% TSA shall be liquefied and the media shall be tempered in a 45 ± 1 °C (113 ± 1 °F) water bath. 1.5% TSA plates shall be room temperature prior to use.

- c) Serial dilutions of MS-2 Coliphage suspension (10^{-1} to 10^{-12}) shall be made using sterile PBS. 10^{-5} to 10^{-12} dilutions shall be plated in triplicate on 1.5% TSA plates. In a sterile tube, 1 mL of diluted MS-2 Coliphage shall be transferred. Then 0.1 mL of *E. coli* host shall be added quickly to ~ 5 mL of melted 1% TSA. The inoculum and media shall be vortexed and poured on TSA plates. The plates shall be rocked to spread inoculum evenly. After the 1% TSA layer has solidified, the plates shall be inverted and incubated at $35 \pm 1^\circ\text{C}$ ($95 \pm 1^\circ\text{F}$) for 18 ± 2 h.
- d) Plates shall be selected that show complete lysis of host cells by the MS-2 Coliphage. The surface of each plate shall be flooded with 3 mL of TSB. The 1% TSA layer shall be gently removed using a cell scraper. The contents shall be poured into two sterile 50 mL centrifuge tubes and the total volume brought to 40 mL with TSB. 0.2 g EDTA and 0.026 g lysozyme shall be added to each tube. The centrifuge tubes shall be incubated at room temperature for 2 h, mixing every 15 min.
- e) After the 2 h incubation, the tubes shall be centrifuged at 9280 *xg* for 5 min, or 2320 *xg* for 20 min, at $20 \pm 1^\circ\text{C}$ ($68 \pm 1^\circ\text{F}$). The resulting supernatant shall be removed while avoiding the pellet. A sterile 47-mm filtration assembly shall be aseptically constructed using a 0.22- μm polycarbonate filter. The filter shall be pretreated with 10 mL of TSB broth just prior to the filtration to minimize MS-2 Coliphage adsorption to the filter. The supernatant shall be filtered.
- f) For long-term storage (greater than 28 d), $1/_{10}$ volume of sterile glycerol shall be added to suspension, dispensed into 1 mL and 3 mL aliquots in cryovials, and stored at $-70^\circ \pm 1^\circ\text{C}$ ($-94 \pm 1^\circ\text{F}$).
- g) The MS-2 Coliphage suspension shall be titrated as in A.8.2.2. The concentration of MS-2 Coliphage should be 10^{10} to 10^{12} PFU/mL.

A.8.2.2 Enumeration of MS-2 Coliphage plaques

- a) A cryogenically frozen *E. coli* host strain shall be thawed. One TSB tube shall be inoculated with 0.1 mL of the stock suspension. The TSB tube shall be incubated at $35 \pm 1^\circ\text{C}$ ($95 \pm 1^\circ\text{F}$) for 18 ± 2 h.
- b) 1% TSA shall be liquefied and the media shall be tempered in a $45 \pm 1^\circ\text{C}$ ($113 \pm 1^\circ\text{F}$) water bath. 1.5% TSA plates shall be room temperature prior to use.
- c) Serial dilutions of MS-2 Coliphage suspension (10^{-1} to 10^{-12}) shall be made using sterile PBS. 10^{-7} to 10^{-12} dilutions shall be plated in triplicate on 1.5% TSA plates. In a sterile tube, 1 mL of diluted MS-2 Coliphage shall be transferred. Then 0.1 mL of *E. coli* host shall be added quickly to ~ 5 mL of melted 1% TSA. The inoculum and media shall be vortexed and poured on TSA plates. The plates shall be rocked to spread inoculum evenly. After the 1% TSA layer has solidified, the plates shall be inverted and incubated at $35 \pm 1^\circ\text{C}$ ($95 \pm 1^\circ\text{F}$) for 18 ± 2 h.
- d) After incubation, plates containing 20 – 200 distinct plaque forming units (PFU) shall be enumerated using a Colony Counter. The MS-2 Coliphage suspension titer shall be calculated by multiplying the number of PFU obtained by the inverse of the dilution factor. The concentration of MS-2 Coliphage should be 10^{10} to 10^{12} PFU/mL.

A.9 Drinking water treatment unit challenge organism suspension preparation

A.9.1 Determination of the concentration of challenge organism

This determination will be based upon the unit flow rates, injection feed pump rate, suspension density, and the final challenge organism concentration for the unit challenge. The suspension shall be of adequate volume to deliver the challenge organism to two complete on/off cycles at each sample point.

Example:

- unit flow rate: 1.0 gpm; duplicate units tested so total of 2.0 gpm (7560 mL/min);
- injection rate: 10 mL/min;
- suspension density: 1×10^9 /mL;
- final concentration: 7.0×10^4 /mL; and
- on/off cycle: 10 min / 10 min (20 min on for two complete cycles).

a) To challenge for 20 min at two 10 min intervals, a total of 200 mL of suspension is needed to challenge 151,200 mL of water (7560 min x 20 min):

- $(7.0 \times 10^4/\text{mL})(151,200 \text{ mL}) = (\text{injection feed conc.})(200 \text{ mL})$; and
- injection feed concentration = 5.3×10^7 /mL.

b) To prepare this from the stock suspension, combine:

- $(200 \text{ mL})(5.3 \times 10^7/\text{mL}) = (\text{mL of suspension density})(1.0 \times 10^9/\text{mL})$;
- mL of suspension density = 10.6 mL; and
- 10.6 mL of suspension to 189.4 mL of PBS.

Once suspension has been made, the suspension shall be mixed using a magnetic stirrer.

A 10-mL aliquot shall be removed from the challenge suspension and set aside for density verification according to *Standard Methods for the Examination of Water and Wastewater*.

A.10 Analysis of influent and effluent samples

A.10.2 Enumeration of MS-2 Coliphage plaques

a) Serial dilutions of the influent and effluent samples (10^0 to 10^{-5}) shall be made using sterile PBS. 10^0 to 10^{-5} dilutions shall be plated in duplicate on 1.5% TSA plates. In a sterile tube, 1 mL of diluted MS-2 Coliphage shall be transferred. Then 0.1 mL of *E. coli* host shall be added quickly to ~ 5 mL of melted 1% TSA. The inoculum and media shall be vortexed and poured on TSA plates. The plates shall be rocked to spread inoculum evenly. After the 1% TSA layer has solidified, the plates shall be inverted and incubated at $35 \pm 1^\circ\text{C}$ ($95 \pm 1^\circ\text{F}$) for 18 ± 2 h.

b) After incubation, plates containing 20 – 200 distinct plaque forming units (PFU) shall be enumerated using a Colony Counter. The MS-2 Coliphage suspension titer shall be calculated by multiplying the number of PFU obtained by the inverse of the dilution factor. Results shall be expressed as the number of PFU/mL.

A.11 Challenge verification

After the appropriate incubation period for MS-2 Coliphage, the plaques shall be counted on all of the density determination plates. The mean number of microorganisms per milliliter for plates with 25 to 250 colonies/plaques shall be calculated. This shall verify that the challenge organism was present in the challenge test water at the optimum concentration before being added to test apparatus.

Attachment 4

UVT Scans of Influent and Effluent Water at high and low UVT

Contact Mr. Bruce Bartley at 734-769-5148 or bartley@nsf.org for a copy of this document.

This is a large pdf file.