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Environmental Technology Verification Protocol

Drinking Water Systems Center

Protocol for Development of Test / Quality Assurance Plans for Validation of Ultraviolet (UV) Reactors

Prepared by

NSF International

Under a Cooperative Agreement with

U.S. Environmental Protection Agency

Contents

Tables

Figures

Abbreviations, Acronyms and Definitions

(See also the EPA's "Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule" for more abbreviations and acronyms common to UV validation.)

ASTM	American Society of Testing Materials
ATCC	American Type Culture Collection
AWWARF	Water Research Foundation (formerly AWWA Research Foundation)
EC	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
HPC	Heterotrophic Plate Count
L	Liter
LIMS	Laboratory Information Management System
mg	Milligram
mL	Milliliter
NaOH	Sodium Hydroxide
ND	Non-Detect
NIST	National Institute of Standards and Technology
nm	Nanometer
NSF	NSF International (formerly known as National Sanitation Foundation)
NTU	Nephelometric Turbidity Unit
NWRI	National Water Research Institute
PFU	Plaque Forming Units
psig	Pounds per Square Inch, Gauge
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
RPD	Relative Percent Deviation
SI	International Systems of Units
SOP	Standard Operating Procedure
TQAP	Test / Quality Assurance Plan
TDS	Total Dissolved Solids
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
UV	Ultraviolet
UVT	Ultraviolet Transmittance

Preface and Scope

This document is an EPA Environmental Technology Verification (ETV) protocol (ETV UV Protocol) for developing a test quality assurance plan (TQAP) for ultraviolet (UV) disinfection. The ETV UV Protocol was developed through the ETV Drinking Water Systems (DWS) Center to clarify vague aspects of the EPA's "Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule" (UVDGM-2006) The ETV UV Protocol describes 1) the objectives of the testing, 2) test procedures, calculations and data reporting, and 3) the quality assurance procedures and quality controls.

The experimental design in this EPA ETV UV Protocol conforms to the sample collection and test procedures described in the EPA's "Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule" (UVDGM-2006) for a single sensor set-point control strategy. This protocol also allows development of data for a "set line" control strategy which is a series of three or more validated set points for a single reactor typically at various flow rates. For a calculated dose strategy, the user should read and follow section 5.10.2 Determining the Validated Dose and Operating Conditions for the Calculated Dose Approach in the UVDGM-2006.

The UV reactor validation produced from testing per this ETV Protocol may be used for any of the following potential applications:

- Determination of the log inactivation credit under the EPA Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) for *Cryptosporidium*, *Giardia* and viruses (although an alternative to MS2 as a test microorganism may be needed to demonstrate greater than 2log reduction of adenovirus see section 3.3.1) in drinking water treatment.
- In the inactivation of microorganisms found in swimming pools, hot tubs and other recreational water facilities (not beach activity).
- In the treatment of water used in bottled water companies.
- To meet international norms of validated dose such as 40 mJ/cm^2.

Should the UV reactor be used in the applications stated above, it is recommended that the materials used in the UV reactor be safe and meet health affects Standards such as NSF/American National Standards Institute (ANSI) Standard 61 for drinking water and 50 for recreational water equipment.

1.0 Equipment Verification Testing Responsibilities

1.1 Verification Test Site

UV dose validation testing shall be performed at a test facility/laboratory such that the testing equipment includes at minimum: injection pumps and ports to introduce the challenge microorganism, the ultraviolet (UV) absorbing compound; a means to remove a disinfectant residual in the feed water; flow rate control and a flow meter upstream and/or downstream of the reactor; and ensure that the water is well mixed before sampling (e.g., static mixers or appropriate number of pipe lengths with good mixing confirmed).

1.2 Roles and Responsibilities

The roles and responsibilities of the validation testing facility, its credentials and experience with UV validation, the vendor, and the EPA ETV Program shall be described.

1.2.1 Validation Test Facility

Validation testing should be performed by an independent third party organization or laboratory. For testing under the Environmental Protection Agency (EPA) Environmental Technology Verification (ETV) Program, an independent third party performing the validation test and prepare the ETV verification report shall conform to the EPA ETV Program Quality Management Plan (QMP).

Examples of independent third parties are those accredited by their respective nation's "accreditation bodies" to ISO Standards for independent third party testing organizations and certifiers: ISO Guide 65 and Standard 17025. ISO Guide 65 contains the general requirements for bodies operating product certification systems. ISO 17025 contains the general requirements for the competence of testing and calibration facilities and organizations.

All testing organizations and laboratories shall follow the procedures and adhere to the requirements of this ETV protocol. The testing organization shall provide in the ETV TQAP and ETV Report the following information:

- Test site description including schematic of hydraulics laboratory and geographic location of the facility;
- Key personnel with identified roles and responsibilities related to the validation and their experience (resumes may be included or provided upon request);
- Present accreditations of the test facility such as conformance to ISO Standard 17025;
- Approximate schedule for testing.

1.2.3 United States Environmental Protection Agency

The EPA provides leadership in the nation's environmental science, research, education and assessment efforts. The EPA works closely with other federal agencies, state and local governments, and Native American tribes to develop and enforce regulations under existing environmental laws. The EPA is responsible for researching and setting national standards for a variety of environmental programs and delegates to states and tribes responsible for issuing permits, and monitoring and enforcing compliance. Where national standards are not met, the EPA can issue sanctions and take other steps to assist the states and tribes in reaching the desired levels of environmental quality. The EPA also works with

industries and all levels of government in a wide variety of voluntary pollution prevention programs and energy conservation efforts. The following are specific EPA roles and responsibilities:

- Quality Assurance (QA) oversight of ETV related activities;
- Technical review and QA oversight of Protocol ;
- Technical and QA reviews and approval of a vendor product test/quality assurance plan (TQAP);
- Direct the performance, at the EPA's discretion, of external technical systems audit(s) during the verification testing;
- Review draft verification reports and statements; and
- Final report approval and clearance for signature by the EPA Laboratory Director.

2.0 Equipment Description

2.1 General Information about the Reactor

The name of the company that makes and/or sells a UV reactor and the UV reactor's make and model designation shall be identified in the TQAP. The company shall provide a description of the UV reactor that includes the following information prior to the start of validation testing:

- The number of UV lamps;
- The number of sensors for the reactor (see information about UV sensors for minimum number of sensors per UV lamps);
- Control strategy (set point etc.);
- Alarms and alarm conditions;
- Provide schematics and drawings with parts and dimensions of the reactor, the sensors, the lamps the quartz sleeve placement and all wetted components; and
- Provide configuration of inlet and outlet (Z or L shapes etc.).

The following information should be provided with each UV reactor:

- Manufacturer/Supplier;
- Type, model, make and number;
- Year of manufacture;
- Maximum flow rate;
- Net weight;
- Volume;
- Voltage and phase;
- Power consumption;
- Lamp Life, ballast type, lamp type, ignition type, lamp operation (constant output or variable); and;
- Power supply unit's name, make and serial numbers.

Information about the UV lamp sleeve:

- Type or model;
- Quartz or other material type;
- Cleaning apparatus, procedure or system; and
- Pressure resistance.

Information about the UV sensor:

- Manufacturer;
- Type / model;
- Measuring field angle;
- Number of sensors per lamp and placement;
- UV emission spectrum measured in Watts at various wavelengths to demonstrate spectral response;
- Germicidal characteristics; and

Signal output range in mA (mV).Reactors shall have no less than one sensor per ten lamps if the UV reactor uses low pressure (monochromatic) lamps and shall have no less than one sensor to one lamp for UV reactors using medium pressure (polychromatic) lamps.

3.0 Validation Testing Design

3.1 Experimental Design

The goals and objectives of the validation shall be stated in the TQAP. An example is the goal of validation to achieve a 3 log inactivation of *Cryptosporidium* (See the Preface of this document for more examples of goals or objectives of a validation). A log inactivation goal of a target pathogen shall be reasonably achieved with the test microorganism. For example, the use of MS2 to achieve a 4 log inactivation of adenovirus would not be reasonable considering the instability of MS2 at the UV dose sufficient to inactivate adenovirus greater than 2 log.

Once the goals and objectives are determined, this ETV Protocol shall be used in creating the experimental design that is described in the accompanying TQAP. This ETV Protocol was derived from the EPA's UVDGM-2006 as desired by the ETV Drinking Water Systems (DWS) Center stakeholders. A TQAP shall be developed based on the goals and objectives of the validation, data quality objectives, the specific make and model of the vendor's reactor for validation and other information requested in this ETV Protocol.

The approach used in the UVDGM-2006 to validate UV reactors is based on biodosimetry that shall be used for this protocol. The biodosimetry approach determines the log inactivation of a challenge microorganism during full-scale reactor testing for specific operating conditions of flow rate, UV transmittance (UVT), and UV intensity (measured by the duty sensor). Then a dose-response equation for the challenge microorganism 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 "Reduction Equivalent Dose (RED)". The RED value is adjusted for uncertainties and biases to produce the validated dose of the reactor for the specific operating conditions tested.

UV reactor validation follows these steps:

1. Assessment of the UV sensors,
2. Collimated beam laboratory bench scale testing,
3. Full scale reactor testing, and
4. Calculations to determine the RED and then to adjust for uncertainty in UV dose, the validated dose.

3.2 UV Sensors Assessment

The test unit shall be evaluated according to the UV sensor requirements in the EPA's UVDGM-2006 prior to any verification testing or provide certificates from an accredited third party organization (see Section 1.2.1 for examples) that the UV sensor met sensor requirements of other third party consensus standards.

All UV intensity sensors (duty and reference sensors) shall be calibrated in accordance with ANSI/NCSL Z540-1-1994 and ISO/IEC 17025-2005 using reference standards traceable to the International System of Units (SI) through the National Institute of Standards and Technology (NIST) or other recognized national laboratory, accepted fundamental or natural physical constants, ratio type of

calibration, or by comparison to consensus standards. Evidence of calibration occurring within twelve months prior to the start of testing shall be provided for all sensors

The technical specifications of the UV sensors and representations of their sensitivity to their germicidal wavelength shall be provided in the TQAP. Sensors shall be germicidal; have a spectral response that peaks between 250 and 280 nm and has less than 10 percent of its total measurement due to light above 300 nm when mounted on the UV reactor and viewing the UV lamps through the water.

During validation testing, duty UV sensor measurements shall be measured to assure that the duty sensors provided by the vendor shall be within ten percent of the average of two or more reference sensor measurements. Duty sensors that do not meet the data quality objective of ten percent shall be replaced by the vendor. The replaced duty sensors shall have to be retested as described below.

The following steps shall be used to check the uncertainty of the duty and reference UV sensors used prior to and at the end of the validation testing:

- Step 1: Water shall be passed through the reactor at the maximum UVT and the maximum lamp power setting to be used during validation testing.
- Step 2: Using two, at a minimum annually, calibrated reference UV sensors; each reference sensor shall be installed on the UV reactor at each port. The UV intensity shall be measured and recorded. Note that should the reference sensors not agree with the calibration certificate-specified measurement uncertainty, they shall be sent back to the vendor for other conforming reference sensors. If the reference sensors are not within 10% of each other, the sensors will be replaced or sent for recalibration.
- The above step shall be repeated using each duty UV sensor. If the UV reference and duty sensors can be rotated, the minimum and maximum sensor readings shall be recorded across the complete range of rotation for each sensor.
- Step 3: Steps 1 and 2 shall be repeated at maximum UVT and lamp power decreased to the minimum level resulting in the minimum intensity expected to occur during validation testing with all lamps turned on.
- Step 4: For a given lamp output and UVT value, the difference between the reference and duty UV sensor measurements shall be:

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

  Where:
  - S_{duty} = Intensity measured by a duty UV sensor,
  - $S_{Avg\ Ref}$ = 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 Collimated Beam Bench Scale Testing

The collimated beam procedure involves placing a sample with the challenge microorganism 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, see equation number one. 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 UVGM-2006 shall be used to conduct the collimated beam bench-scale testing and produce a UV dose-response curve for the MS2 and other acceptable test organisms (see section 3.3.1 on acceptable test microorganisms). As Appendix C is guidance, the following sections describe the details of the collimated beam testing.

Collimated beam tests shall be performed using two water quality conditions: the highest UVT and the lowest UVT used in the full scale reactor test. At least one collimated beam test should be conducted on each day of full-scale reactor testing using either the highest or lowest UVT.

3.3.1 Test Microorganisms (challenge)

The UVDGM-2006 allows for a variety of different target microorganisms for the estimation of log reduction for *Cryptosporidium* and viruses. MS2 (ATCC 15597-B1) phage shall be used as the test microorganism for the target pathogens, *Cryptosporidium* and *Giardia*. For other target pathogens such as adenovirus, an alternative test microorganism may need to be used as MS2 is not suitable to demonstrate a 4 log reduction at a 186 mJ/cm^2 dose. MS2 was selected for UV validation in drinking water based on the following:

- The MS2 phage was selected as it is very reproducible between and within laboratories and it has a known record of ease of use and culturing.
- MS2 phage also has a well known quality control history and levels of acceptance are established such as the quality control boundaries in the National Water Research Institute/American Water Works Association Research Foundation "Ultraviolet Disinfection Guidelines for Drinking Water and Water Reuse," Second Edition, May 2003, Fountain Valley, CA (NWRI/AWWARF, 2003).
- The MS2 phage is recommended for reactors to validate to a dose such as 40mJ/cm2 commonly referenced in Europe and several USA States.

Other test microorganisms may be used only if a peer and drinking water stakeholder review accepts the alternative test microorganism. Quality control and assurance data shall be included in any documentation submitted for selecting an alternative challenge microorganism for UV validation. The stability of the test organism used by the testing facility shall be established over at least a three week period. At least one study on the variability associated within and between laboratories shall be conducted and data submitted before consideration.

3.3.2 Test Conditions

The collimated beam test shall be performed at the minimum and maximum UVT test condition. At least one collimated beam test shall be performed during each day of the test. See Section 3.4.4 for the minimum and maximum UVT test condition. The water used for the collimated beam bench scale testing shall be the same water used for the full scale reactor testing.

UV doses shall cover the range of the targeted dose. Table 3-1 illustrates an example of the samples and target doses for the MS2 phage plus a control (zero (0) UV dose) that will be produced in the study.

Table 3-1. Example Target Dose for Collimated Beam Test Using MS2

Sample	Log Inactivation	Target UV Doses (mJ/cm^2) *Depending on objective, other target doses may be appropriate such as for viruses*
Low UVT #1	0 (control)	0
Low UVT #2	1.0	20
Low UVT #3	1.5	30
Low UVT #4	2.0	40
Low UVT #5	3.0	60
Low UVT #6	4.0	80
High UVT #1	0 (control)	0
High UVT #2	1.0	20
High UVT #3	1.5	30
High UVT #4	2.0	40
High UVT #5	3.0	60
High UVT #6	4.0	80

3.3.3 Test Apparatus

The challenge microorganism's UV dose-response should be measured using a low pressure (LP) collimated beam apparatus. An example of a LP collimated beam apparatus is provided in Appendix V of the EPA's UVDGM-2006.

3.3.4 Frequency of Testing

The collimated beam bench-scale tests shall be performed for the challenge microorganism during each day of testing in duplicate. A collimated beam bench scale test shall be performed using the highest and one using the lowest UVT condition during the entire test. Should the full scale testing be done in one day, the highest and lowest UVT collimated beam bench scale tests shall be completed that day. All collimated beam tests for a given test condition (highest or lowest UVT) shall be run in duplicate.

3.3.5 Accuracy of Instruments

The accuracy of spectrophotometer measurements of A_{254} shall be determined using NIST traceable potassium dichromate UV absorbance standards and holmium oxide UV wavelength standards on each day of the test. The measurement uncertainty of the spectrophotometer shall be ten percent or less from the standards. UV absorbance of solutions used to zero the spectrophotometer shall be verified using reagent grade organic-free water certified by the supplier to have zero UV absorbance. The spectrophotometer shall be calibrated within one month preceding the start of testing.

Radiometers shall be calibrated according to the following procedure to ensure that the UV intensity is measured with an uncertainty of eight percent or less at a ninety five percent (95%) confidence level:

1. The radiometers used in the collimated beam tests shall come from the radiometer manufacturer with a certified uncertainty of eight percent or less at a 95% confidence level at the intervals suggested by the manufacturer.

2. The accuracy of the radiometer used to measure the UV intensity shall be verified at least at the beginning and the end of each collimated beam test session using a second radiometer.
3. The two radiometers shall have overlapping 95% confidence intervals of their respective measured readings and should read within five percent of each other. If the two radiometers do not read within five percent of each other or have overlapping 95% confidence intervals, a third radiometer shall be used to identify which radiometer is out of specification. The two radiometers with overlapping 95% confidence intervals of their respective measured readings or have readings within five percent of each other shall be used. If none of the radiometer readings match, at least two of them are likely out of calibration.
4. If the above criteria are met, the average radiometer measurement shall be used in calculations. If the criteria are not met, the radiometers shall be sent for recalibration.
5. The radiometers shall also be checked to be sure that the irradiance measurement does not differ by more than five percent before and after UV exposure.

Radiometers shall also be calibrated by the radiometer manufacturer within one year of the start of testing.

3.3.6 Collimated Beam Procedure

The laboratory shall collect a one liter sample from the influent sampling port of the biodosimetry test stand for collimated beam testing. One collimated beam test shall be performed in duplicate for each day of the test. For the entire test, one collimated beam test shall be performed using the highest and lowest UVT test water.

Should a situation develop where a different batch (propagation) of challenge microorganisms is used, UV dose-response curves shall be generated for each batch or propagation of the challenge microorganisms.

Personnel shall perform the collimated beam tests with all standard safety precautions such as wearing of goggles and latex gloves. The skin of personnel shall be shielded from exposure to UV light.

The protocol for culturing MS2 and measuring its concentration will follow a double agar overlay method described in Standard Methods 9224.

For collimated beam testing of a water sample containing challenge microorganisms, the laboratory shall follow this procedure:

1. Measure the A_{254} 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 detector within the radiometer shall be placed at the same distance as the meniscus of the 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 block of the light 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 ºC (for up to 24 hours). (Should the laboratory perform a stability test to assess holding time and test water on infectivity of the phage, then the samples could be held for a longer tine for analysis.) Plate each sample in triplicate, enumerate the plaques on each plate, calculate the concentration of phage in the starting sample and report each replicate plating.
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 five percent 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 which should be similar to the 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 collimated beam tests measurements shall produce:

- UV Dose in units of mJ/cm^2;
- Concentration of microorganisms in the petri dish prior to UV exposure (N_o) 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 UV dose delivered to the sample is calculated using the following equation:

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

where:

D_{CB} = UV dose (mJ/cm^2)
E_s = Average UV intensity (measured before and after irradiating the sample) (mW/cm^2)
P_f = Petri Factor (unitless) (see Appendix C in UVDGM-2006 for procedure)
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)
t = Exposure time (s)

To control for error in the UV dose measurement, the variances of the terms in the UV dose calculation shall meet the following criteria (see Appendix C in UVDGM-2006 for details on calculation of variances)

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

A regression analysis shall be used to develop each UV dose-response curve. The regression analysis to derive an equation that best fits the data shall involve forcing the fit through the origin. The laboratory shall try both linear and a quadratic equations to determine the best fit of the data to a linear model. The test facility or laboratory shall then evaluate the equation's goodness-of-fit. The differences between the measured UV dose values and those predicted by the equation should be randomly distributed around zero and not be dependent on UV dose. The test facility / laboratory shall establish the "goodness of the fit" using standard statistical tests, such as examining the p-statistics for the regression coefficients. The regression coefficient, R^2, is acceptable if it is 0.9 or greater and for "r" +/- 0.95 or greater. All data shall be within the 95% confidence intervals of the regression curve. The curve shall not be used to predict values outside the range of the data. For MS2, the data shall be evaluated to and found to meet the quality control requirements described in the NWRI/AWWARF, 2003.

3.3.7 Collimated Beam Data Uncertainty

Since the collimated beam data shall be fit to a linear or a polynomial regression, a 95-percent confidence interval (U_{DR}) shall be calculated:

$$U_{DR} = t * [SD/ \text{UV Dose}_{CB}] \text{ X } 100\% \qquad \text{(Eq. 2)}$$

where:

U_{DR} = Uncertainty of the UV dose-response fit at a 95-percent 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
t = t-statistic at a 95-percent confidence level for a sample size equal to the number of test condition replicates used to define the dose-response.

The UV dose-response curve for specific UVT of the test water shall be from the data produced during each day of the full scale testing, (combined dataset) and used in the calculation of uncertainty.

Using Eq. 2 for calculating U_{DR}, for each CB test before continuing, the data quality objective is not to exceed 30 percent at the UV dose corresponding to 1-log inactivation of the challenge organism. Should the U_{DR} value be greater than 30 percent, the uncertainty shall be added to the total uncertainty of validation as described in Section 6.

Analysis of regression coefficients indicates whether or not UV dose-response curves developed using different water samples can be combined. The data from different days and different UVT shall not be combined. In order for the UV dose-response curves to be combined, differences between the regression coefficients should not be statistically significant at a 95-percent confidence level. If differences in the coefficients are statistically significant, the reason for this difference shall be documented. Differences between measured UV dose-response curves for different water samples could indicate one or more of the following:

- The UV dose-responses of different batches of the challenge microorganism differ. In this case, the UV dose-response curve specific to each cultured batch of the challenge microorganism should be used to assess UV dose delivery for the validation test conditions using that batch.

- Interferences due to water quality; In this case, the test facility / laboratory shall mitigate the cause of the interference or account for the interference when assessing UV dose delivery for the validation test conditions.
- Errors calculating the UV dose delivered by the collimated beam apparatus.

If there are two or more UV dose-response curves from the same day of testing, the data shall not be combined and the curve resulting in the most conservative (lowest) UV dose shall be used for calculating RED values.

3.4 Full Scale Testing to Validate UV Dose

3.4.1 Evaluation, Documentation and Installation of Reactor

Prior to the start of validation testing, the testing facility shall confirm that the vendor met the description and documentation requirements for equipment required in Section 2.0 of this document. The UV reactor and the reactor inlet and outlet connections shall be installed at the laboratory in accordance with the vendor's installation and assembly instructions. The piping shall be inspected to ensure compliance with the vendor's specifications. The physical integrity of the UV reactor and the test train shall be verified before testing.

3.4.2 Lamp Positioning and Burn-In Period

UV lamps shall undergo a minimum burn-in period of one hundred hours. The lamps shall be repositioned in the reactor with the lamp with the highest output closest to the sensor as recommended in Section 5.4.7 of UVDGM-2006.

The following procedure shall be used to determine the UV lamp output at the lowest and highest ballast setting.
1. Install a lamp within a lamp sleeve located at the position nearest to the single UV sensor.
2. Pass water through the reactor at a constant (+/- 10%) flow rate, temperature and UVT.
3. With only the lamp under evaluation on, record the measured UV intensity.
4. Repeat the test for each lamp at each ballast setting.

To determine the rank of each lamp's output, use the following process:
1. Calculate the average of the measured UV intensity of all lamps at the highest ballast setting.
2. Divide each lamp's measured UV intensity at the highest ballast setting by the average of the measured UV intensity of all lamps at the highest ballast setting.
3. The result is a ranking of each lamp at the highest ballast setting.
4. Repeat steps 1 through 3 above for lamps at the lowest ballast setting and determine the ranking of each lamp at the lowest ballast setting.
5. To determine the final ranking of the lamps, take the rank of each lamp at each ballast setting to determine an average ranking for each lamp's UV output.

For the full-scale reactor testing, the test facility / laboratory shall install the lamp with the highest average output closest to the duty UV sensor. The remaining lamps shall be distributed randomly within the reactor with respect to lamp intensity.

3.4.3 Test Hydraulics: Equipment, Water Source and UV Absorbing Chemical(s)

Per Section 5.4 of the UVDGM-2006, the testing equipment needed for the conduct of a full scale reactor validation "should include:

- Injection pumps and ports to introduce the challenge microorganism;
- The UV absorbing compound, and, if needed, a disinfectant residual quenching agent into the feed water;
- Rate-of-flow control and a flow meter either upstream or downstream of the reactor; and
- A strategy to ensure that the water is well mixed before sampling (e.g., static mixers or appropriate number of pipe lengths with good mixing confirmed.

At least one ninety (90) degree elbow shall be fitted between the influent sample port and the inlet of the UV reactor for testing to simulate worst case hydraulic conditions. At least one ninety (90) degree elbow shall be fitted between the outlet of the UV reactor and the effluent sample port to simulate worst case hydraulic conditions. The test facility shall provide a detailed schematic of the validation facilities. The configuration of the inlet and outlet piping and other hydraulics of the test shall be fully described in the TQAP and final report.

The water source for test shall be of potable water quality preferably municipal drinking water. The water shall be de-chlorinated using activated carbon or a chemical that shall not affect the viability of the test microorganism. Proof shall be provided of viability integrity before use of chlorine neutralizing chemicals. See Section 4.4.2 for guidance on establishing stability and viability of test organisms. When validating UV reactors using polychromatic output lamps, the quenching agent should not affect by more than ten percent the spectral UVT from 200 to 400 nm when compared to not using a quenching agent.

The chemical SuperHume™ should be used to lower the UV transmittance. An alternative chemical may be used to lower UVT, if when evaluated, meets the criteria in UVDGM-2006 including but not limited to absorption of UV over the spectrum pertinent to the type of UV lamp (at 254nm for low pressure) and has no effect on the viability of the challenge microorganism. The UVT reduction chemical shall be added to the supply tank before each run or set of runs. The supply tank shall be well mixed using a recirculating pump system.

By reference, the test equipment and design shall conform to the specification described in the following sections of UVDGM-2006:

- Microorganisms and UV absorbing chemicals shall be mixed through the cross-section of the influent pipe – Section 5.4.3; and
- Sampling ports and points for microorganisms – Section 5.4.4.

3.4.4 Test Conditions for UV Intensity Set-point

This validation testing must determine the operating conditions under which the reactor delivers the required UV dose for treatment credit (see 40 CFR 141.720(d)(2)). The operating conditions shall include at a minimum the flow rate, UV intensity as measured by a UV sensor, and UV lamp status.

This approach relies on one or more "setpoints" for UV intensity that are established during validation testing to determine UV dose. During operations, the UV intensity as measured by the UV sensors must

meet or exceed the setpoint(s) to ensure delivery of the required dose. Reactors must also be operated within validated operation conditions for flow rates and lamp status [40 CFR 141.720(d)(2)]. In the UV Intensity Setpoint Approach, UVT does not need to be monitored separately. Instead, the intensity readings by the sensors account for changes in UVT. The operating strategy can be with either a single setpoint (one UV intensity setpoint is used for all validated flow rates) or a variable setpoint (the UV intensity setpoint is determined using a lookup table or equation for a range of flow rates).

The manufacturer determines the UV intensity setpoint for their reactor and provides the information to the test facility and it is described in the TQAP. The first test condition involves reducing UVT until UV intensity measured by the UV sensor is equal to the UV intensity setpoint. The second test condition involves testing at high UVT but reducing power until the UV intensity measured by the sensor is equal to the UV intensity setpoint

UVT shall be adjusted using SuperHume™. The testing laboratory/facility may conduct a scan to determine the absorbance curve for SH prior to its use.

A single setpoint validation test requires a minimum of three test conditions including a reactor control test as indicated in Table 3-2.

Table 3-2. Test Conditions for Validation

Test Conditions[a]	Flow Rate	UV Transmittance	Lamp Power
Condition 1	Maximum Design	UVT of sensor setpoint [b]	100%
Condition 2	Maximum Design	Maximum of test water (> 95%)	reduced to UVT of sensor setpoint [b]
Condition 3 (reactor control)	Maximum Design	Maximum of test water (> 95%)	Turned off

a) Each test condition except the reactor control will be run in duplicate.

b) The manufacturer determines the UV intensity setpoint for their reactor and provides the information to the test facility and it is described in the TQAP. The first test condition involves reducing UVT until UV intensity measured by the UV sensor is equal to the UV intensity setpoint.

3.4.5 Preparation of the Challenge Microorganisms

The challenge microorganisms, MS2 used to validate UV reactors shall be cultured and analyzed by the microbiology laboratory as specified in Standard Methods 9224 *Standard Methods for the Examination of Water and Wastewater* (APHA et al. current edition). The specific edition of Standard Methods used shall be stated in the report. Protocols for culturing the challenge microorganism and measuring its concentration shall be defined and based on published and peer-reviewed methods.

Propagation will result in a highly concentrated stock solution of essentially monodispersed phage whose UV dose-response follows second-order kinetics with minimal tailing. Over the range of reduction equivalent dose (RED) values demonstrated during validation testing, the mean UV dose-response of the MS2 phage stock solution should lie 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 millijoules per centimeter squared (mJ/cm2), the prediction intervals of the data shown in Appendix A of the UVDGM-2006 are represented by the following equations"

$$Upper\ Bound: \log I = -1.4\times10^{-4}\times UV\ Dose^2 + 7.6\times10^{-2}\times UV\ Dose$$
$$Lower\ Bound: \log I = -9.6\times10^{-5}\times UV\ Dose^2 + 4.5\times10^{-2}\times UV\ Dose$$

To assure that the challenge microorganism concentrations are stable over the holding time between sampling and completion of the assays, tap water shall be 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 test facility may test the filtered challenge water for the following parameters to assess potential sources or causes of challenge microorganisms' instability (see Section 4.4.2):

- Total chlorine;
- Free chlorine;
- UV_{254} ;
- UVT;
- Total iron;
- Total Manganese;
- Turbidity;
- Total coliform; and
- Heterotrophic plate count (HPC).

3.4.6 Conduct Testing – Measuring UV Dose

During full-scale reactor testing, the reactor is operated at each of the test conditions for flow rate, UVT, and lamp power as described in Section 3.4.4.

The following steps shall be taken to assure meeting data quality objectives:

- Steady-state conditions shall be confirmed before injecting the challenge microorganism. Confirmation of steady-state involve monitoring UV sensor measurements and the UVT to assure the test water and reactor meet the test conditions such as UV sensor intensity reading of eighty percent.
- The challenge microorganism shall be injected into the flow upstream of the reactor to achieve at least a concentration of $1 \text{ X } 10^5$ pfu/mL.
- Sample taps shall remain open over the duration of the test.
- Samples shall be collected in accordance with standards of good practice as defined by *Standard Methods* Section 9060 (APHA et al.1998).
- Sampling shall begin after establishing continuous flow, injection of challenge organisms and / or Superhume™ and after a minimum of three times the hydraulic retention time of the reactor has been achieved. The inlet and outlet samples shall be collected as close as possible to the hydraulic retention time between the inlet and outlet sampling ports. The inlet and outlet samples shall be spaced evenly as possible of a minimum of 10 minutes.
- Sample volumes for assessing the challenge microorganism concentrations in the influent and effluent shall be no less than 15 milliliter (mL).
- Samples shall be collected in bottles that have been cleaned and sterilized.
- Collected samples shall be stored on ice, within a cooler, in the dark and then delivered to the microbiological lab. Analysis shall be performed within 24 hours of the time the sample was collected.

The following measurement and recording shall be taken to assure meeting data quality objectives:

- The flow rate through the reactor, UV sensor measurements, UVT measurements;
- Water chemistry grab samples shall be collected once per test condition either before or after the challenge organism samples are collected. Samples for temperature, pH, *E. coli*, and HPC shall be collected at the influent and effluent locations, and samples for iron, manganese, turbidity and residual chlorine shall be collected at the influent location.
- The UVT as measured by the UV spectrophotometer with each influent sample; and
- The electrical power consumed by the UV reactor.

The test shall be repeated if the flow rate, UV intensity, lamp power, or UVT changes by more than the error of the measurement over the course of sampling.

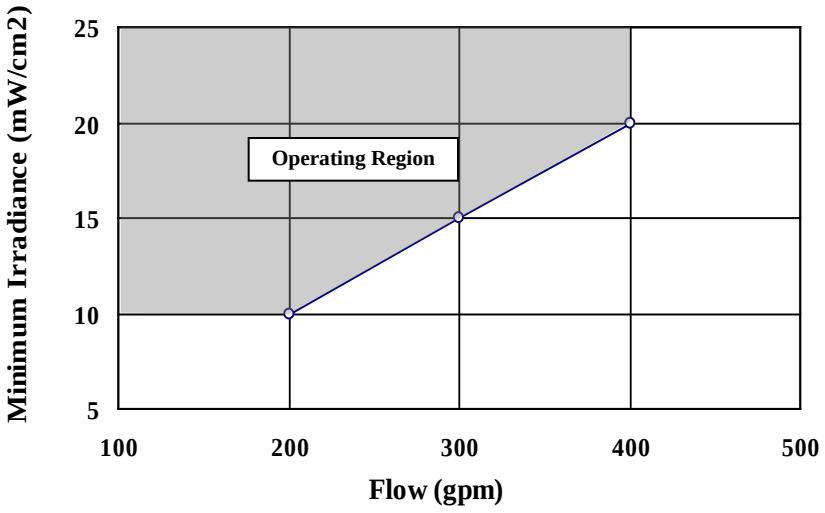
The following measurements and recordings shall be taken to assure data quality objectives: Influent and effluent water samples shall be collected and analyzed for pH, E.coli, and HPC only at the beginning and the end of the test. Influent samples only shall be collected and analyzed for iron, manganese, turbidity and residual chlorine at the beginning and end of a test.

3.4.7 Test Conditions for UV Intensity Set-line or Multiple Set Points

The testing described for determining the control strategy of a UV intensity set-point, shall be used to establish multiple set points or a set line (three or more set points) control strategy. A set point is a combination of a maximum flow rate - minimum irradiance measurement that delivers the required UV dose. A set line is three or more set points.

For example, with three set point conditions validated for each microorganism, i.e. maximum flow rate - minimum irradiance combinations and with corresponding UV doses, the data could be presented in a summary table and then graphed to show a setline. Figure 3-1 shows an example of a setline graph. As shown in the graph, a combination of maximum flow rates and minimum irradiance define an operating region, which ensures the required UV dose is delivered. The operating region is above and to the left of the

Figure 3-1. Example Set Line



4.0 Laboratory Operations Procedures

4.1 Introduction

This protocol specifies procedures that shall be used to ensure the accurate documentation of UV reactor performance. Careful adherence to these procedures and to the analytical procedures shall result in verifiable performance data.

4.2 Analytical Methods

All laboratory analytical methods for water quality parameters are listed in Table 4-1.

Table 4-1. Analytical Methods for Laboratory Analyses

Parameter	Method	Reporting Limit	Lab Accuracy (% Recovery)	Lab Precision	Hold Time	Sample Container	Sample Preservation
Temperature	SM 2550[0]	-	-	-	(2)	NA	None
pH	SM 4500-H^+B	NA	±0.1 S. U. of buffer	±0.1 S. U.	(2)	NA	None
Total Coliform/ *E. coli*	SM 9222B or SM 9223B	1 CFU/100mL	NA	NA	30 hours	125 mL sterile Nalgene	None
Iron	EPA 200.7	20 µg/L	70-130	≤ 30%	180 days	125 mL polyethylene	nitric acid to pH <2
Manganese	EPA 200.7 EPA 200.8	1 µg/L	70-130	≤ 30%	180 days	125 mL polyethylene	nitric acid to pH <2
Turbidity	SM 2130B	0.1 NTU[(3)]	95-105		(2)	NA	None
MS2	Top agar overlay	1 PFU/mL	NA	NA	(4)	125 mL sterile Nalgene	None
Absorbance (UV_{254})	SM 5910B	0.000 Absorbance/cm	60-140	≤ 20%	48 hours	125 mL amber glass	None
Residual Chlorine	SM 4500-Cl D	0.05 mg/L	90-110	≤ 10%	(2)	NA	None
Heterotrophic Plate Count	SM 9215B	1 CFU/mL	NA	NA	24 h	125 mL Sterile Nalgene	None

(1) SM = Standard Methods
(2) Immediate analysis required
(3) NTU = Nephelometric Turbidity Unit
(4) Analyze immediately, or store at 4C in the dark for up to 24 hours only if necessary
(5) h = hours

4.2.1 Sample Processing, and Enumeration of MS2:

MS2 bacteriophage sample processing and enumeration will follow Method 9224 *Standard Methods for the Examination of Water and Wastewater* (APHA et al. current edition) .

4.2.2 Percent UVT measurements:

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

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

Spectrophotometer measurements of A_{254} shall be verified using NIST-traceable potassium dichromate UV absorbance standards and holmium oxide UV wavelength standards. The UV spectrophotometer internal quality assurance and quality control (QA/QC) procedures shall NOT be used to verify calibration. UV absorbance of solutions used to zero the spectrophotometer shall be verified using reagent grade organic-free water certified by the supplier to have zero UV absorbance.

The measurement uncertainty of the spectrophotometer must be ten percent or less. To achieve this goal, the following procedure shall be used from UVDGM-2006:

1. Verify that the spectrophotometer reads the wavelength to within the accuracy of a holmium oxide standard (typically ± 0.11 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 ± 0.004 cm^{-1} 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.

If the spectrophotometer provides biased readings, the measurements shall be corrected to account for that bias, or another instrument with measurement uncertainty of ten percent or less shall be used.

4.3 Analytical QA/QC Procedures

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

- pH – Three-point calibration (4, 7, 10) of the pH meter used to give the reportable data shall be conducted daily using traceable buffers. The accuracy of the calibration shall be checked daily with an independent (separate lot and preferably separate source from the calibration buffers) pH buffer. The pH reading for the buffer shall be within $\pm$ 0.1 SU of the true value. The precision of the meter shall be checked daily using duplicate synthetic drinking water samples. The duplicate samples shall be within $\pm$ 0.1 SU.

- Temperature – The thermometer used to give the reportable data shall have a scale marked for every 0.1ºC. The thermometer is calibrated yearly using a Hart Scientific Dry Well Calibrator Model 9105.

- Total chlorine – The calibration of the chlorine meter shall be checked daily using a DI water sample (blank), and three QC standards. The measured QC standard values shall be within ten percent of their true values. The precision of the meter shall be checked daily by duplicate analysis of synthetic drinking water samples. The RPD of the duplicate samples shall be less than ten percent.

- Turbidity – The turbidimeter shall be calibrated as needed according to the manufacturer's instructions with formazin standards. Accuracy shall be checked daily with a secondary Gelex standard. The calibration check shall give readings within 5% of the true value. The precision of the meter shall be checked daily by duplicate analysis of synthetic drinking water samples. The RPD of the duplicate samples shall be less than ten percent.

4.4 Sample Handling

All samples not immediately analyzed shall be labeled with unique identification numbers. These identification numbers shall be entered into a Laboratory Information Management System (LIMS), and shall appear on the lab reports for the tests. All challenge organism samples shall be stored in the dark at 4 ± 2 °C and processed for analysis within twenty-four hours or within the holding time established by stability tests described in Section 4.4.2.

4.4.1 Power Measurements

Voltmeters, ammeters, and power meters used to measure (1) ballast and UV equipment input voltage, and (2) consumed current and power, must have evidence of being in calibration (e.g., have a tag showing that it was calibrated).

4.4.2 Full Scale Test Controls

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

- *Reactor controls* – influent and effluent water samples shall be collected with the UV lamps in the reactor turned off. The change in log concentration from influent to effluent should correspond to no more than 0.2 $\log_{10}$.
- *Reactor blanks* – a sample of influent and effluent water shall be collected with no addition of challenge microorganism from the flow passing through the reactor at least once on each day of testing. The concentration of challenge microorganisms shall be quantified. Should the challenge microorganism exceed a 0.2 $\log_{10}$ concentration, the plumbing associated the testing facility, shall be disinfected. Another reactor blank sample shall be collected and enumerated after disinfection and neutralization of the disinfectant. The reactor blank is considered acceptable for challenge testing if the challenge organism does not exceed 0.2 $\log_{10}$ concentration.
- *Trip controls* – one sample bottle of challenge microorganism stock solution shall travel with the stock solution used for validation testing from the microbiological laboratory to the location of reactor testing and back to the laboratory. The change in the log concentration of the challenge microorganism in the trip control should be within the measurement error of 0.2 $\log_{10}$ concentration
- M*ethod blanks* – a sample bottle of sterilized reagent grade water that undergoes the challenge microorganism assay procedure shall be analyzed. The concentration of challenge microorganism with the method blank shall be non-detectable, according to *Standard Methods for the Examination of Water and Wastewater* (APHA et al. 1998).
- *Stability samples and testing* – Laboratories shall 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 unless the laboratory analyzes samples within 12 hours of collection. Otherwise, all laboratories shall follow the stability analyses of this section. The influent and effluent samples shall be collected at low and high UVT. The challenge microorganism shall be added to achieve a concentration of 1,000 plaque forming units (pfu)/L in the samples containing test water at the lowest and highest UVT. A sample will be analyzed immediately (called time 0) and then 24 hours, 48 hours and 96 hours after time 0. All analyses will be performed in triplicate. The challenge microorganism concentrations in the stability samples shall not vary by more than +/- 0.2 $\log_{10}$ of the time 0

sample. The testing facility may conduct stability assessment of its test water prior to conducting any validation test.

4.4.3 Flow Rate

During validation testing, the variability or precision of flow rate measurements should be less than or equal to five percent**.** The measurement uncertainty of the flow meter shall be verified by the catch and weigh method or for larger flow rates that cannot be "caught and weighed" by an alternative method, such as a calibrated pitometer or second calibrated flow meter, that can provide the required flow meter calibration and uncertainty data..Calibration of flow rate shall be completed prior to and within six months of the start of testing.

4.5 Documentation

All laboratory activities shall be thoroughly documented using lab bench sheets and the LIMS laboratory reports.

The laboratory/test facility shall be responsible for maintaining all documentation. Lab bench sheets shall be used to record all water treatment equipment operating data in ink as described in Section 6.2 Each page shall be labeled with the project name and number. Errors shall have one line drawn through them and this line shall be initialed and dated.

Any deviations from the approved final TQAP shall be thoroughly documented at the time of inspection and in the verification report.

5.0 Quality Assurance Project Plan

5.1 Introduction

A Quality Assurance Project Plan (QAPP) shall be prepared as part of the TQAP for the EPA ETV verification. Procedures shall be used to ensure data quality and integrity will be specified in the QAPP and TQAP. Careful adherence to these procedures shall ensure that data generated from the verification testing provides sound analytical results that can serve as the basis for the performance verification.

This section outlines steps that shall be taken by the laboratory/testing facility to ensure that data resulting from verification testing is of known quality and that a sufficient number of critical measurements are taken.

5.2 Quality Assurance Responsibilities

A number of individuals shall be responsible for test equipment operation, sampling, and analysis QA/QC throughout the verification testing. Primary responsibility for ensuring that these activities comply with the QA/QC requirements of the TQAP rests with the supervisors of the individual laboratories.

The laboratory/testing facility QA/QC staff shall review the raw data records for compliance with QA/QC requirements. The laboratory/testing facility staff shall check one hundred percent of the raw data records against the reported results in the LIMS reports.

5.3 Data Quality Indicators

The data obtained during the verification testing must be of sound quality for conclusions to be drawn on the treatment equipment. For all verification activities, data quality parameters must be established based on the proposed end uses of the data. These parameters include five indicators of data quality: representativeness, accuracy, precision, statistical uncertainty, and completeness.

5.3.1 Representativeness

Representativeness refers to the degree to which the data accurately and precisely represent the conditions or characteristics of the parameter represented by the data, or the expected performance of the reactor under normal use conditions. Representativeness shall be ensured by executing consistent sample collection protocols, including timing of sample collection, sampling procedures, and sample preservation. Representativeness shall also be ensured by using each analytical method at its optimum capability to provide the most accurate and precise measurements possible.

5.3.2. Accuracy

Accuracy is a measure of the deviation of the analytical value from the true value. Since true values for samples can never be known, accuracy measurements are made through analysis of certified standards or QC samples of a known quantity.

Accuracy shall be maintained through the following items:

- Maintaining consistent sample collection procedures, including sample locations, timing of sample collection, and sampling procedures;

- Calibrated instruments; and
- Laboratory control samples (e.g., method blanks, duplicates, matrix spikes, matrix spike duplicates, and performance evaluation samples).

Recoveries for spiked samples shall be calculated in the following manner:

$$\text{Percent Recovery} = \frac{100*(SSR - SR)}{SA}$$

where: SSR = spiked sample result
SR = sample result
SA = spike amount added

Recoveries for laboratory control samples are calculated as follows:

$$\text{Percent Recovery} = \frac{100*(\textit{Found Concentration})}{\textit{True Concentration}}$$

For acceptable analytical accuracy, the recoveries must be within control limits. The laboratory's minimum acceptable accuracy for each parameter is listed in Table 4-1.
The accuracy of the benchtop chlorine, pH, and turbidity meters shall be checked daily during the calibration procedures using certified check standards.

5.3.3 Precision

Precision refers to the degree of mutual agreement among individual measurements and provides an estimate of random error. Precision shall be measured through duplicate sample analysis. One sample per batch shall be analyzed in duplicate for the total dissolved solids (TDS) and alkalinity analyses. To check the precision of the benchtop chlorine, pH, and turbidity meters, duplicate synthetic drinking water samples shall be analyzed daily.

The standard deviation and the relative standard deviation recorded from sample analyses shall be reported as a means to quantify sample precision. The percent relative standard deviation should be calculated in the following manner or equivalent method such as relative percent deviation (RPD) :

$$\%\ \text{Relative Standard Deviation} = \frac{S(100)}{X_{average}}$$

where: S = standard deviation
$X_{average}$ = the arithmetic mean of the recovery values.

Standard Deviation is calculated as follows:

$$\text{Standard Deviation} = \sqrt{\frac{\sum_{i=1}^{n}\left(X_i - X\right)^2}{n-1}}$$

where: X_i = the individual recovery values
X = the arithmetic mean of then recovery values
n = the number of determinations.

For acceptable analytical precision under the verification testing program, the percent relative standard deviation for drinking water samples must be less than 30%.

Precision of the duplicate analyses may be measured by use of the following equation to calculate RPD:

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

where:

S_1 = sample analysis result; and

S_2 = sample duplicate analysis result.

Acceptable RPD values for each parameter are given in Table 4-1.

5.3.4 Statistical Uncertainty

Statistical uncertainty of the triplicate challenge organism counts shall be evaluated using Microsoft® Excel 2003 to calculate the 95% confidence intervals. The following formula shall be employed for confidence interval calculation:

$$\text{confidence interval} = \overline{X} \pm t_{1-\frac{\alpha}{2}} \left(S/\sqrt{n}\right)$$

where: $\overline{X}$ is the sample mean;
S is the sample standard deviation;
n is the number independent measures included in the data set;
t is the Student's t distribution value with n-1 degrees of freedom; and
α is the significance level, defined for 95% confidence as: 1 - 0.95 = 0.05.

The uncertainty of the collimated beam data calculations shall be performed according to Section 3.3.6. The acceptable uncertainty is thirty percent.

5.3.5 Completeness

Completeness refers to the amount of data collected from a measurement process compared to the amount that was expected to be obtained. Completeness refers to the proportion of valid, acceptable data generated using each method. This portion of the required data for the selected test plan shall be reported at the conclusion of each testing period.

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. The following chart illustrates the completeness objectives for performance parameter and/or method based on the sample frequency:

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.

Retesting may be required if the completeness objectives are not met.

The following are examples of instances that might cause a sample analyses to be incomplete:

- Instrument failure;
- Calibration requirement not being met; or
- Elevated analyte levels in the method blank.

5.5 Data Validation and Reporting

To maintain good data quality, specific procedures shall be followed during data validation, and reporting. These procedures are detailed below.

5.5.1 Data Validation

For the analytical data:

- The laboratory/testing facility staff shall review calculations and inspect laboratory logbooks and data sheets to verify accuracy of data recording and sampling;
- The laboratory/testing facility QA/QC department shall verify that all instrument systems are in control and that QA objectives for accuracy, precision, and method detection limits have been met; and

- The laboratory/testing facility QA staff shall review the raw data records for compliance with QC requirements and check one hundred percent of the data against the reported results from the LIMS reports.

Should QC data be outside of control limits, the analytical laboratory supervisor shall investigate the cause of the problem, and discussion of the problem shall be included in the final report. Depending on the severity of the problem, the data in question may be flagged, or not reported.

5.5.2 Data Reporting

The data to be reported shall be the feed and treated water microorganism counts, log reductions, and the water chemistry data. The QC data, such as calibrations, blanks and reference samples shall not be reported, but shall be kept on file for inspection upon audit of the facility.

5.6 Testing Inspections

QA staff shall conduct an audit of the laboratory during testing to ensure compliance with the test procedures and requirements of this Protocol and the developed TQAP. Any variances shall be reported to the laboratory/testing facility QA staff.

6.0 Data Management, Analysis, and Reporting

6.1 Data Management and Analysis

All operational and analytical data shall be gathered and included in the Final ETV Report. The data shall consist of results of analyses and measurements and QA/QC reports.

The data management system for this verification involves the use of the laboratory/testing facility LIMS computer system, spreadsheet software and manual recording of system operating parameters.

6.2 Work Plan

The following is the work plan for data management:

- Laboratory personnel shall record equipment operation, water quality and analytical data by hand on bench sheets.
- All bench sheet entries shall be made in water-insoluble ink.
- All corrections on the bench sheets shall be made by placing one line through the erroneous information. Any corrections shall be dated and initialed by the lab personnel making the correction.
- Pertinent information from the bench sheets shall be entered into the LIMS system. When the test is complete, a preliminary report shall be generated. The preliminary report shall be reviewed by the manager of any laboratory that entered data. Once the preliminary report is approved, a final laboratory report shall be generated and given to ETV staff.

The database for this verification testing program shall be set up in the form of custom-designed spreadsheets. Pertinent data from the LIMS reports shall be entered into the appropriate spreadsheets. All recorded calculations shall also be checked at this time. Following data entry, the spreadsheet shall be printed out and the printout checked against the LIMS report.

6.3 Report of Equipment Testing and Performance

The report shall be issued in draft form for review prior to final publication. The reports shall be prepared by the laboratory/testing facility and shall consist of the following:

- Introduction;
- Description and Identification of Product Tested;
- Procedures and Methods Used in Testing;
- Results and Discussion, including QA/QC discussion; and
- References.

6.3.1 Report Introduction

- Study objectives; and
- Data quality objectives.

6.3.2 Report – Reactor and Equipment Information

The minimum contents of the report introduction shall include:

General information about the UV Reactor:

- Specifications for the UV sensor port indicating all dimensions and tolerances that impact the positioning of the sensor relative to the lamps; and
- Specifications giving the window material, thickness, and UV transmittance as appropriate.

Lamp specifications

- Lamp manufacturer and product number;
- Electrical power rating;
- Electrode-to-electrode length;
- Spectral output of new and aged 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);
- Mercury content if applicable; and
- Envelope diameter.

Lamp sleeve specifications

- Technical description including sleeve dimensions;
- Material; and
- UV transmittance at 254 nm .

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, head loss, and pressure rating of the reactor;
- Assembly and installation instructions;
- Electrical requirements, including required line frequency, voltage, amperage, and power;
- Operation and maintenance manuals that include cleaning procedures, required spare parts, and safety requirements; and
- Safety requirements.

6.3.3 Report Results, Calculations and Discussion

The collimated beam dose response data shall be reported using tables and graphs. Table 6-1 is an example of collimated beam data reports.

Table 6-1. Example UV Dose – Response Measurements from Collimated Beam Tests

	Lowest UVT(1)				Maximum UVT				
	Replicate # 1		Replicate # 2			Replicate # 1		Replicate # 2	
Target UV dose (mJ/cm^2)	N as pfu/mL	Log N	N as pfu/mL	Log N	Target UV dose (mJ/cm^2)	N as pfu/mL	Log N	N as pfu/mL	Log N
0					0				
20					20				
30					30				
40					40				
60					60				
80					80				

(1) Low UVT is determined based on lowering the UVT such that the duty sensor on the test unit reads the target intensity for the test as specified by the manufacturer.

One UV dose-response curve shall be developed for each UVT condition tested (one high UVT and one low UVT). The procedure for developing the UV dose response curve is as follows:

1. For each UVT test condition and replicate, plot log N (pfu/mL) vs. UV dose (mJ/cm^2) to identify a common N_o as the intercept of the curve at UV dose = 0. Table 6-1 shows an example table used to collect the collimated beam data A separate figure and equation is developed for each UVT condition (lowest and highest). If more than one day of testing is performed and there are additional sets of data for one or more UVT condition, then separate figures and equations are developed for each dataset.

   The guidance in the UVDGM-2006, Appendix C. Section C.3 item 1 and footnote 2 shall be used to determine the N_0 value at the y intercept. The calculation of N_0 uses all data points including the measured log N at the zero (0) UV dose. The calculated N_0 value shall be used for all subsequent calculation (see Step 2) in accordance with UVDGM-2006 recommendation.

2. Calculate log I 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)
   N = Concentration of challenge microorganisms in the petri dish after exposure to UV light (pfu/mL)

3. Plot UV dose as a function of log I for each UVT test condition.

4. Use regression analysis to derive an equation that best fits the data, forcing the fit through the origin. The force fit through the origin is used rather than the measured value of No, 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 the error carry through.

The UVDGM-2006 recommends combining UV dose response curves whenever possible. For each microorganism (e.g. MS2,) there will be a minimum of four data points for each UV dose (low UVT in duplicate, maximum UVT in duplicate). The data for both low and high UVT shall be evaluated to determine if they can be combined into one data set. The UVDGM-2006 guidance (Appendix C Section C.3 and C.50) provides information on the statistics and procedures to be used to evaluate if data sets can be combined. Additional discussion is also presented in Sections 3.3.6 and 3.3.7 in this protocol.

In general, the data on observed test conditions (flow rate UVT, lamp power and UV sensor intensity measurements), measured influent and effluent MS2 concentrations including triplicate results and UV duty sensor checks shall be presented in tabular form. Tables 6-2, 6-3, and 6-4 show examples of the tabular data that is collected from the validation tests.

Table 6-2. Flow Rate, UVT, Lamp Power, and UV Sensor Data during Test Runs

Test Condition	Replicate Run Number	Flow Rate (gpm)	UVT (%)	Lamp Power (%)	Sensor Reading (mW/cm^2)
1	**1**				
1	**2**				
2	**1**				
2	**2**				
3	**1**				
3	**2**				
Continue Table for as many conditions and the condition replicate runs as needed.					

Table 6-3. Influent and Effluent Challenge Microorganism Concentrations

Test Condition - Replicate	Influent Log Concentration			Effluent Log Concentration		
	Replicate #			Replicate #		
	1	2	3	1	2	3
1-1						
1-2						
2-1						
2-2						
3-1						
3-2						
Continue Table for as many conditions and the condition replicate runs as needed.						

Table 6-4. Reference UV and Duty Sensor Checks

Before/after Testing	UVT (%)	Lamp Power (%)	Duty Sensor (mW/cm^2)	Refer. Sensor #1 (mW/cm^2)	Refer. Sensor #2 (mW/cm^2)
Before	Low	100			
Before	High	Low			
After	Low	100			
After	High	low			

6.3.3.1 RED Calculations

The Reduction Equivalent Dose (RED) results shall be averaged for each test condition and evaluated to identify the minimum value.

For each test condition replicate (i.e., each influent and effluent sample taken at approx. the same time - three sets collected over ten minutes), the log inactivation (log I) will be calculated using the following equation:

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

where:

N_o = challenge microorganism concentration in influent sample in pfu/mL, N = Challenge microorganism concentration in corresponding effluent sample (pfu/mL).

Next the RED, in mJ/cm^2, for each test condition replicate (i.e., each influent and effluent sample taken at approx. the same time - three sets collected over ten minutes) shall be determined using the measured log inactivation (log I) and the collimated beam test UV dose-response curves. If individual dose-response curves developed on the same day of testing cannot be combined, the curve resulting in the lowest RED values shall be used.

The individual replicate RED values shall be averaged to produce one RED for each test condition. From these average values, the minimum RED shall be selected and used in the validated dose calculation. RED values determined for each test condition and replicate will be summarized in a table

with the calculated average and the standard deviation. Table 6-5 shows an example table format for presenting the RED data for MS2.

Table 6-5. Log Inactivation and RED Results

Test Condition	UVT (%)	Log I			RED (mJ/cm^2)				
		Replicate #			Replicate #			Avg.	SD_{RED}
		1	2	3	1	2	3		
1	Low								
2	High								
3	Low								
4	High								
5	Low								
6	High								

6.3.3.2 Deriving the Validation Factor

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 validated dose shall be determined from dividing the RED by a validation factor (VF) to quantitatively 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

6.3.3.3 RED Bias and Factor

The RED bias factor is a correction factor that accounts for the difference between the UV sensitivity of the target pathogen and the UV sensitivity of the test microorganism. The RED bias factor will be derived from the procedures described in Section 5.9.1 of the UVDGM-2006.

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 EPA 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, shall be used to determine U_{Val} in calculating the validated dose.

Per Figure 5.4 in EPA's UVDGM-2006, any of the following equation 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 in:

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.

The EPA's UVDGM-2006 defines how the above uncertainties are calculated.

The QC requirement that the duty sensor measurements should be within 10% of the average of two or more reference sensors eliminates the need to calculate the U_S factor per the UVDGM-2006, Section 5.4.4. The U_{DR} factor calculation is shown in Section 3.3.8 of this TQAP. The UVDGM also shows the formula and calculations for U_{DR} in Appendix C, section C4.

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

1. Calculate the average and standard deviation of RED values for each test condition (typically at least 3 – 5 replicate pairs are generated for each test condition).

2. Calculate the uncertainty of the setpoint RED using:

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

where:
RED = Average RED value measured for each test condition
SD_{RED} = Standard deviation of the RED values measured for each test condition
t = t-statistic for a 95-percent confidence level defined as a function of the number of replicate samples and assuming n-1 degrees of freedom.
Note: the UVDGM has error in the description of this statistic. The t-statistics should be for n-1 degrees of freedom, not for the n-degrees of freedom shown in the UVDGM.

3. Select the highest U_{SP} from all test conditions for calculating the VF.

After establishing the U_{Val}, the VF is calculated (see above equation). The validated dose shall then be calculated as follows:

Validated dose = RED/VF.

The final report and summary statement, shall present the validated dose contingent on these operating conditions:

- The UV intensity as measured by the duty sensors must be greater than the UV intensity set-point to achieve the validated dose;
- The flow rate must be equal to or less than the test condition flow rate; and

The lamp status must be equivalent to the status of the lamps during the test

Attachment 1:

Reactor Schematic Diagrams and Technical Manual