Disinfection Performance Testing of High-Efficiency Ultraviolet Water Treatment Chamber

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1. Introduction

When introducing a disinfection product which incorporates new technology, it is essential that the methods and testing used to quantify the performance of those systems follow protocols which are technically sound and which are accepted by the scientific and technical community within the industry. These protocols must be applied precisely and rigorously in order to ensure accurate, repeatable results. The data generated from experiments following these protocols must be carefully analyzed using proper statistical approaches. These analyses must account for variances due to changes in the product's performance as it ages, tolerances of measurement equipment, and the natural uncertainties associated with the dose-response characteristics of microorganisms. All of these items must be properly addressed for the rating of the product to be accurately determined.

This paper describes the methodology used to verify the rated performance of NeoTech Aqua's "D" series of ultraviolet reactors for water purification. Also included is a description of the protocol which was followed for testing the units, a description of the equipment used for testing, the results obtained during the water disinfection characterization tests, and a description of the method for determining the rating of the reactors from the data collected. The information in the sections below details how the experiments and analysis of the results performed address the requirements for an accurate product rating.

This is the second in a series of three white papers describing the design and performance of the NeoTech Aqua ReFlex™ treatment chamber. The first describes in detail the theoretical basis for the very high efficiency demonstrated by the chamber. The third paper describes how this chamber design leads to some highly desirable operational advantages beyond just energy ad cost reduction.

2. Experimental Methods

The testing at NeoTech Aqua was performed following the testing methodology described in the United States Environmental Protection Agency's Ultraviolet Disinfection Guidance Manual (UVDGM) [1], Section 5, "Validation of UV Reactors." The UVDGM describes the recommended protocol in great detail. As it is quite lengthy, it will only be summarized here. The main steps in the protocol are:

1. Select a challenge microorganism,
2. Perform bench-scale static tests, using a collimated beam apparatus (including NIST-traceable diagnostics) to establish a dose-response curve for that specific sample of the challenge microorganism,
3. Perform full scale flow tests to measure the log inactivation of the same sample of challenge organisms by injecting them upstream of the UV reactor, then measuring the log reduction of samples taken downstream of the UV reactor versus control samples taken upstream of the UV reactor. Samples are analyzed

for a given set of test conditions including flow rate, UV transmittance of the water, lamp status, and UV irradiance as measured by UV sensors,

4. Estimate the "reduction equivalent dose" (RED), by combining the results of the static and flowing tests to generate the normalized dose applied to the challenge microorganisms inside the UV reactor, and
5. Adjust the RED to account for any factors affecting the experimental results in order to generate the "validated dose."

Detailed information regarding each of these steps as NeoTech Aqua has performed them is provided in the following paragraphs.

2.1 Selection of the Challenge Organism

Bacillus subtilis spores (ATCC 6633) have been chosen as the challenge organism. This is the variety recommended in the EPA protocol, and is readily available. The spores are prepared as described in Appendix A, section A.3 of the UVDGM, with the following exceptions. A commercially prepared sporulation media [HiMedia M1018 Sporulation Broth, (SB), VWR catalog # 95020-298] for *B. subtilis* is used in place of the Schaeffer's medium. Tryptic Soy Agar (TSA, BD Difco) is used instead of Columbia agar in steps requiring an agar medium. Incubation periods in SB range from 21 to 32 days. More reliable growth and higher spore concentrations have been obtained using this media combined with an extended incubation period, compared to the results using Schaeffer's media. Sonication has been eliminated from the protocol as it did not prove to influence results.

2.2 Static Tests to Establish Dose-Response

The static tests to establish the dose-response curve for the challenge organism are performed with a collimated beam apparatus in the manner described in Appendix C of the EPA UVDGM. The collimated beam static test system designed and constructed by NeoTech Aqua is slightly different than the one described in the UVDGM. The unit design and these modifications (described in Section 3.1. below) are intended to increase the accuracy and the throughput of the collimated beam testing.

A static test is performed in parallel with each individual flowing test, using samples taken directly from the flowing test skid's sample holding tank within 5 minutes of performance of the flowing test. This ensures that the samples for the static test are as identical as possible to those used for the flowing test.

Duplicate samples are taken at each treatment dose specified in the static test protocol. Testing is performed at typically 10 dose levels ranging from 15 to 150 mJ/cm^2 during each static test. The samples are plated on TSA pour plates using serial dilutions to establish the control inoculation and the inactivation level for each treatment dose. The pour plates are incubated for three days, at 37^oC, at which time the colonies are manually counted. The data is then recorded and analyzed.

2.3 Flowing Tests to Measure System Performance

Individual tests of the UV reactors are performed using a test skid constructed specifically for that purpose. The skid's construction and features are described below in Section 3.2.

Prior to introduction into the holding tank, the sample water is filtered using a combination pleated and granular activated carbon filter, followed by a mixed bed ion exchange unit. This sample water is tested for chlorine and 254 nm UV transmittance (UVT) prior to use. The maximum allowable chlorine level for testing is <0.02 ppm. This level is at the measurement threshold of the instrumentation available, and is more than an order of magnitude less than the concentration required to show measurable inactivation of spores over a one hour treatment time [2]. The UVT is dependent on the desired value for the test. The samples are introduced into the holding tank, and the water is agitated for a minimum of 5 minutes, after which the tank control samples and static test samples were taken. The flowing test is run as soon as possible after these samples were taken, and no longer than 5 minutes later. Five to six different flow rates are typically sampled for each individual test. One control sample and three test samples are taken at each flow rate. The entire individual test typically requires 3-4 minutes, ensuring that the conditions at each flow rate, for each test, are as similar as possible. Following each test, the test skid and the UV reactor are disinfected, following an established and verified protocol.

As with the static test, the flowing test samples are plated on TSA pour plates using serial dilutions to establish the control inoculation and the inactivation level for each flow rate. The pour plates are incubated for three days at 37^{o}C, at which time the colonies were counted manually and the data recorded and analyzed. The data from the static test and the flow test are then combined, per the UVDGM protocol, to create the "reduction equivalent dose" (RED) for the UV reactor and flow rates under test.

2.4 Estimation of the "Reduction Equivalent Dose" (RED)

The "reduction equivalent dose" (RED) is a computed dose which accounts for test-to-test differences in the susceptibility to UV of the challenge microorganisms. The natural variation in the log reduction of the challenge microorganism can vary by 1 – 2 logs, even in the most tightly controlled experiment using samples from identically grown and processed populations of challenge organisms. An example of this variation for *B. subtilis* spores is shown in the figure below, which was reproduced from the UVDGM (p. A-6).

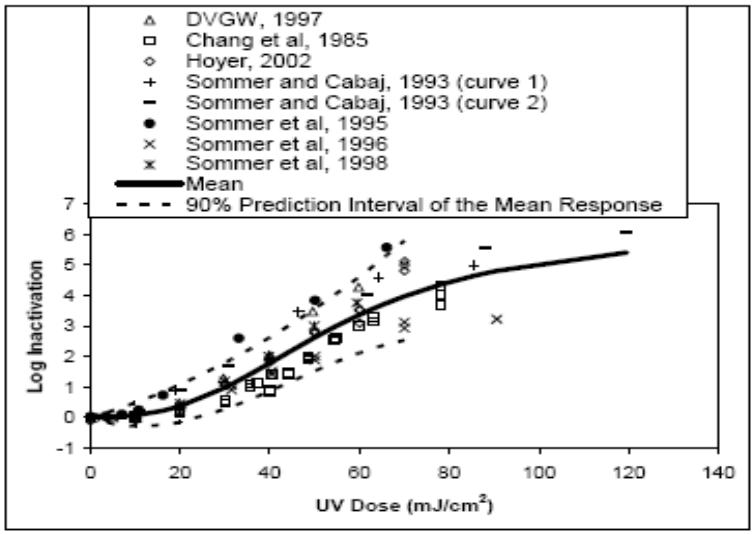

Figure 1. UV dose-response of B. Subtilis spores from UVDGM.

This natural variation, shown in the chart above, requires that the log reduction data from each flowing test be normalized to the corresponding log reduction data from an accompanying static test performed using a portion of the total sample from the same group of challenge microorganisms.

The RED for a specific sample of challenge microorganisms is calculated using the following steps:

1. Perform the collimated beam test, measure and plot the log reduction at the different doses, then fit a curve to that data to produce a dose-response curve for that particular sample.

2. Perform a flowing test in the UV reactor under test at different flow rates using other members of the same sample of challenge organism, and measure the log reduction at each flow rate.

3. Take the log reduction at each flow rate and determine the UV dose required to produce the same reduction on the collimated beam test dose-response curve, either graphically or by using the equation fitted to the dose-response data. The result is the RED for the UV reactor under test at those individual flow rates.

2.5 Adjustment of RED to Determine the "Validated Dose".

The "validated dose" for a particular pathogen or organism can be calculated by dividing the RED by the validation factor (VF). The validation factor accounts for differences between the UV sensitivity of the challenge organism and that particular pathogen, as well as any uncertainties due to the individual characteristics of different experimental setups and monitoring equipment. In the UVDGM, the validation factor takes the form

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

where:
VF = Validation Factor,
B_{RED} = RED bias factor (accounts for difference between pathogen and challenge organism), and
U_{Val} = Uncertainty of validation expressed as a percentage (accounts for other uncertainties).

The UVDGM provides a lengthy discussion of the calculation of the validation factor, which encompasses a wide range of operating conditions. Not all of these factors are utilized in all cases. As an example, for general disinfection equipment, B_{RED} is not used, since the system is not designed for a particular pathogen. Instead, a generally accepted disinfection dose (e.g., 30 mJ/cm^2) is used as a design target.

U_{Val} is dependent on the uncertainties present in the collimated beam test and the test of the unit itself. Figure 5.4 of the UVDGM (page 5-39) provides guidance for the calculation of U_{Val}. Referring to this figure, for the tests performed at NeoTech Aqua, the sensor uncertainty and the uncertainty of the fit of the dose-response curve are negligible, so U_{Val} is equal to only the uncertainty associated with the flowing tests themselves. The effect of the validated dose calculation is incorporated into the data presented in Section 4.

3. Test Equipment and Description of UV Reactors Tested

3.1 Static Test System (Collimated Beam Apparatus)

The static test system designed and constructed by NeoTech Aqua is patterned after the collimated beam apparatus described in Appendix C of the UVDGM, with modifications made to improve the accuracy and repeatability compared to that of the apparatus described therein.

The apparatus defined in the UVDGM calls for the illumination of the sample to be performed in a Petri dish with a magnetic stirrer. The following are inherent issues with this approach which can lead to inaccuracies in the test:

1. The Petri dishes are made from a material which does not transmit UV.

2. Petri dishes have a large diameter relative to the typical UV lamp footprint, and they have vertical walls which can block some of the impinging UV.

3. The magnetic stirrer pellet produces a shadow which causes portions of the sample to receive a significantly lower UV dose than that received by the rest of the sample.

4. The magnetic stirrer pellet would need to be sterilized, and aseptically transferred from sample to sample, at which time adherence of cells or contamination could alter results.

The above factors lead to a non-uniform dose being applied to the sample. In the UVDGM, there is a recommended adjustment called the "Petri factor" to correct for these issues (see Section II.E.).

The NeoTech Aqua static test system replaces the Petri dish and magnetic stirrer with small (5 ml) polypropylene sample cups to address the issues above. The polypropylene sample cups are 92% transmissive at 254 nm, are narrower than the UV lamp footprint, and have sloped sides. Figure 2 is a photograph of a sample cup.

Figure 2. Polypropylene collimated beam test cup

All of these features help to eliminate the shadowing and non-uniformity that occurs near the edges of a Petri dish and underneath the magnetic stirrer pellet. The sample cups are bagged and gamma-ray sterilized by the manufacturer to minimize the possibility of contamination.

Care is taken to ensure that the challenge organism samples used for static testing do not settle., The testing is performed very quickly (see below), and the samples are agitated sufficiently during the process to prevent re-clumping or settling, so there is no need for the magnetic stirrer.

Other improvements allow the static test system to process samples much faster than the system described in Appendix C of the UVDGM. The small width of the sample cups ensures a low level of irradiance variation, even with the sample cups very close to the UV source. Peak-to-peak irradiance variation across these sample cups has been measured at <8% in the static test system with the sample cup only 12 cm away from the aperture of the lamp box (see Figure 3). This high uniformity at such a short distance

leads to higher UV irradiance on the sample. Higher irradiance allows for a significant increase in throughput compared to the system described in the UVDGM.

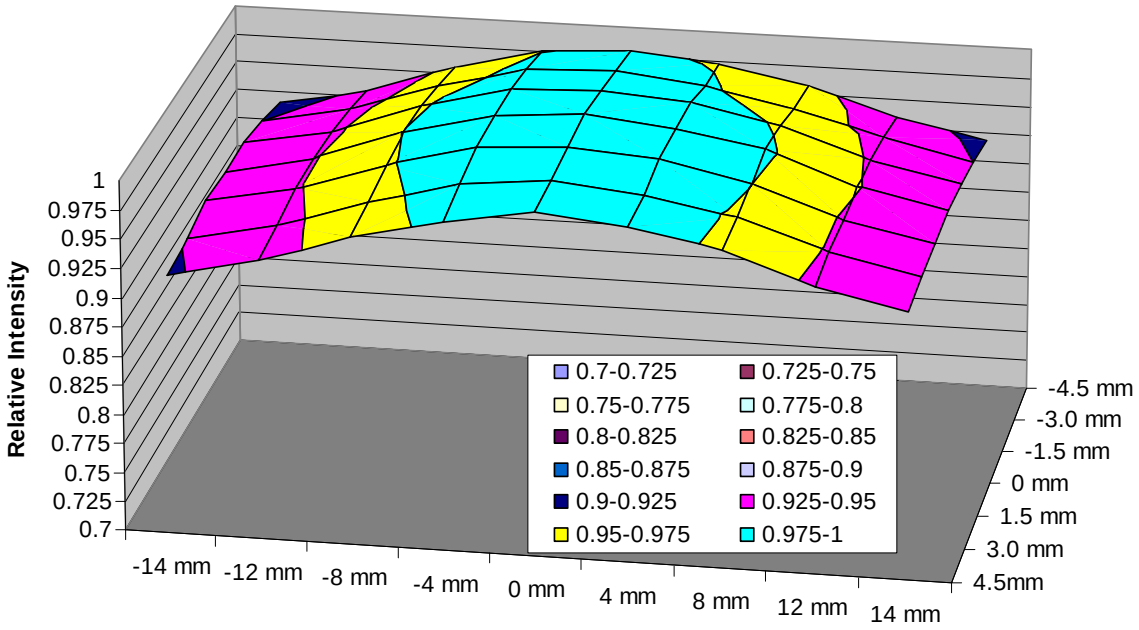

Figure 3. Irradiance at the surface of the polypropylene collimated beam test cup in the NeoTech Aqua static test system

The small length of the sample cups allows for location of the fiber optic cable and cosine corrector of the NIST-traceable spectrometer (Ocean Optics 2000 series) immediately next to the sample cup. As a result, the spectrometer is able to measure the UV irradiance applied to the sample in real time. This is an improvement over the procedure recommended in Appendix C of the UVDGM, in which irradiance is measured before and after the sample is illuminated, and increases the speed and accuracy of static testing.

The static treatment system is operated by a computer which monitors the UV irradiance from the spectrometer, closes a shutter to "turn off" the UV when the programmed dose is reached, and records the measured dose for each sample. This automated static test system is proven to provide a dose which is accurate and repeatable to well within 5% of the desired dose entered by the operator.

These improvements result in a system which can rapidly and precisely generate highly accurate dose-response measurements for use in calculating the RED of a UV reactor. Since this system overcomes the issues raised with the collimated beam apparatus described in the UVDGM, its contribution to the "validation factor" described above is negligible. A photo of the system is shown in Figure 4.

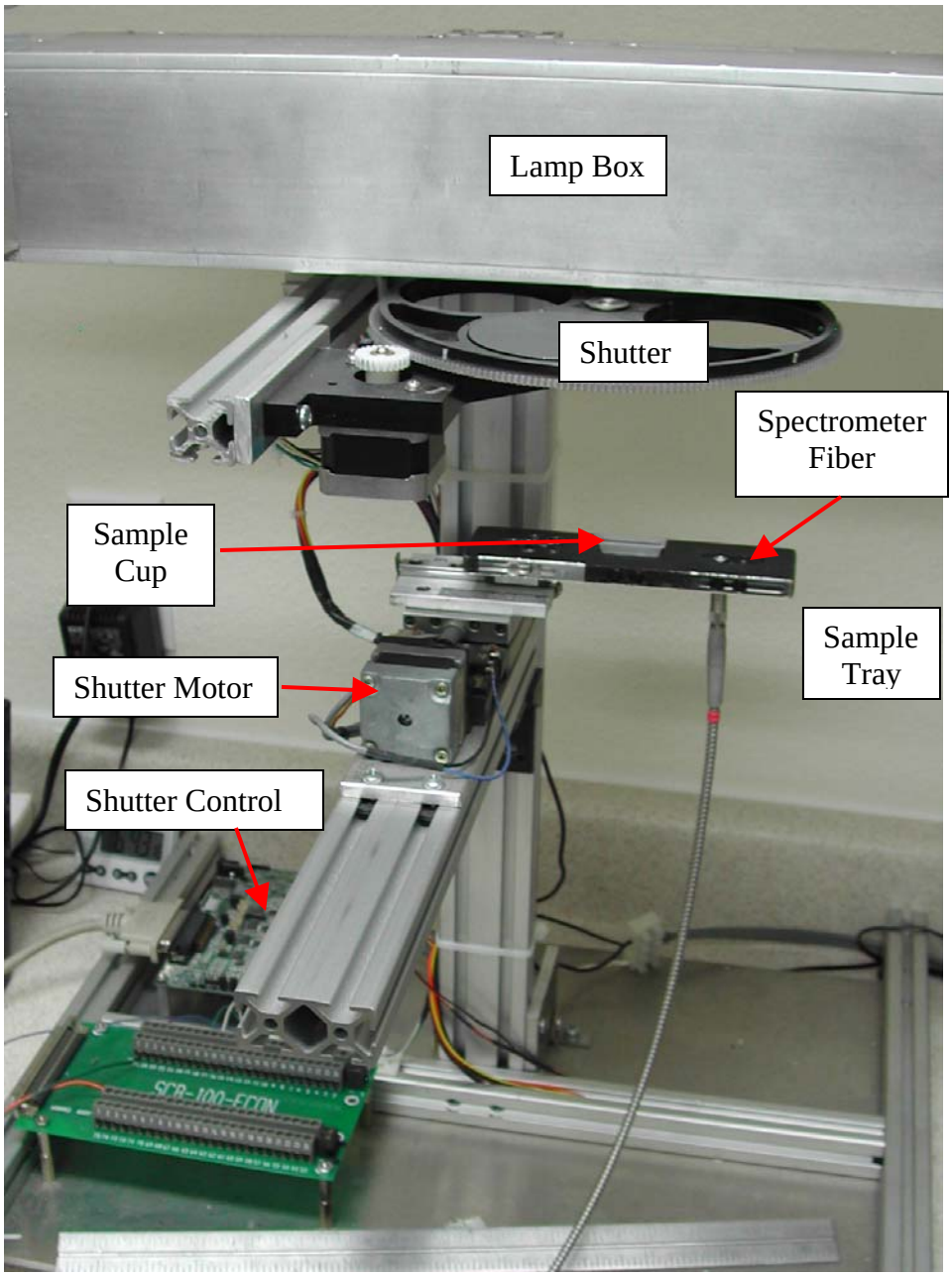

Figure 4. NeoTech Aqua Static Test System for performing high throughput collimated beam tests.

3.2 Flowing Test Skid

Figure 5. NeoTech Aqua 170 GPM Flowing Disinfection Test Skid.

The flowing disinfection test skid designed and constructed by NeoTech Aqua is capable of performing single pass flowing tests using up to 300 gallon samples and flow rates of 2 GPM to over 170 GPM. The components of the skid, including the pump, gauges, and flow meters are tri-clamp type sanitary models and are made with food grade or higher purity materials. Tri-clamp type sanitary connections and fittings are also used for all piping and hoses to minimize the possibility of contamination. Millipore ESP family sanitary sample ports are used to take all flowing test samples. These sample ports are installed as close to the unit as possible. There are 90º elbows between the unit and the sample ports to ensure that no significant UV light reaches the points at which samples are taken.

The skid is designed such that test can be prepared and run within 5 minutes. This eliminates the need for static mixers or other components to keep the challenge microorganism uniformly mixed in the water, because the test can be performed before the microorganisms can settle or clump.

3.3 System Diagnostics

The diagnostic equipment used to record the data reported is listed below:

Static Test System (Collimated Beam Apparatus):
Ocean Optics 2000 Spectrometer
Windows PC running custom static test system operating code
Cary 3E UV-Visible Spectrophotometer (for measuring sample UVT)
ExTech Instruments ExStik CL 200 chlorine meter

Flowing Test System:
Flow meters: Blancett FloClean Sanitary type 0.75-7.5 GPM (model B16A-105A-0BA), 3-30 GPM (model B16A-108A-0BA), or 15-180 GPM (model B16A-115A-0BA)
Cary 3E UV-Visible Spectrophotometer (for measuring sample UVT)
ExTech Instruments ExStik CL 200 chlorine meter
Winters AAA Sanitary 150 psi pressure gauge
Winters AAA Sanitary 200 psi pressure gauge
NeoTech Aqua UV Intensity Monitor (NIST traceable photodiode-based 254 nm narrow-band UV diagnostic integrated into UV reactor)

4. Experimental results

4.1. Dose-Response of Challenge Organisms Compared to Published Static Test Results

Figure 1 below is a typical dose-response curve for the colonies of *B. subtilis* grown at NeoTech Aqua. This curve is within the bounds of the 90% prediction interval shown in the UVDGM and reproduced in Figure 1 above. The maximum variation of less than 0.5 logs seen in the data within each test and from test to test indicates that the growth and testing techniques are sound and reproducible.

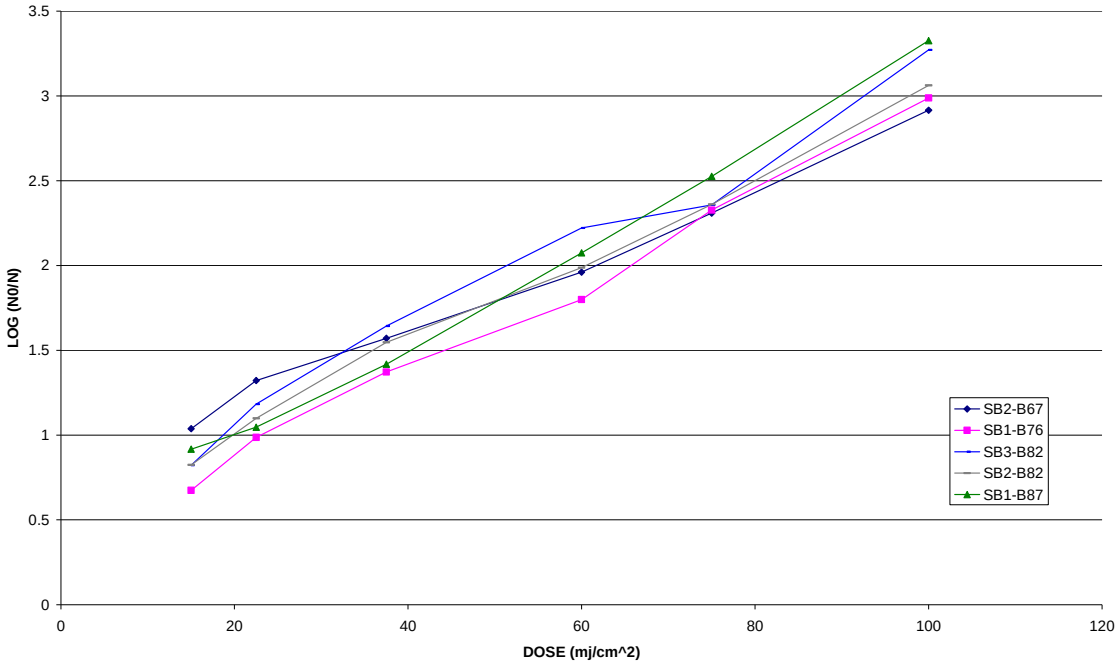

Figure 6. Typical static test dose-response curve for B. subtilis ATCC 6633 grown at NeoTech Aqua and tested using NeoTech Aqua static test system.

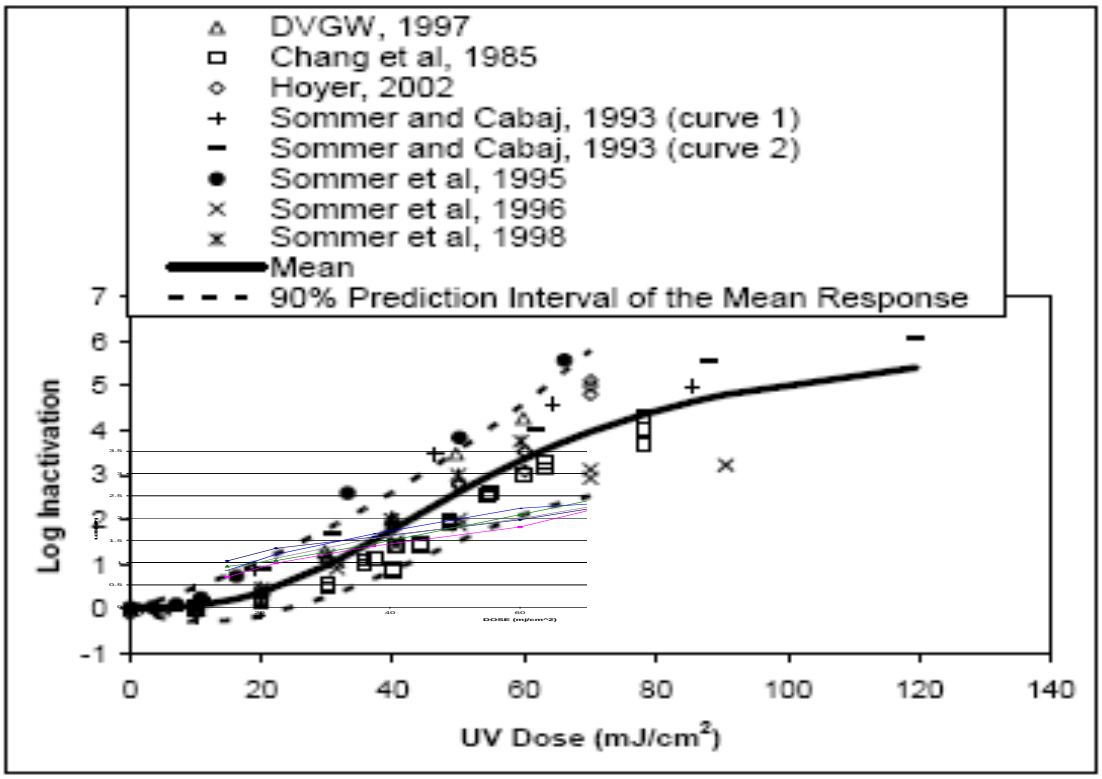

Figure 7. Data from Figure 6 overlaid onto the UVDGM dose-response curve. The data fall within the 90% Prediction Interval of the Mean Response range of that figure.

4.2 System RED Based on Dose-response of Challenge Organisms

The reduction equivalent dose (RED) as a function of the flow rate for the various NeoTech "D" series of UV treatment systems is shown in the figures below for 95% UV transmittance water. The same tests are performed at 99% UVT and at 90% UVT as well. These data are representative samples from over 200 distinct tests taken in house. The data sets include the calculated end of lamp life (EOLL) validated dose as described in the UVDGM.

These data were all generated by NeoTech Aqua. Pertinent third party data and a comparison of results from third party data with that generated by NeoTech Aqua are found in Section 4.3. below. The validated dose lines on these plots are the basis for the published performance ratings for each of these systems. Note that there is no in-house data for the D438 system. The disinfection capability of that system is beyond what can be tested with our in-house test system. The test data for that system were generated at the National Sanitary Foundation's test facility in Ann Arbor, Michigan.

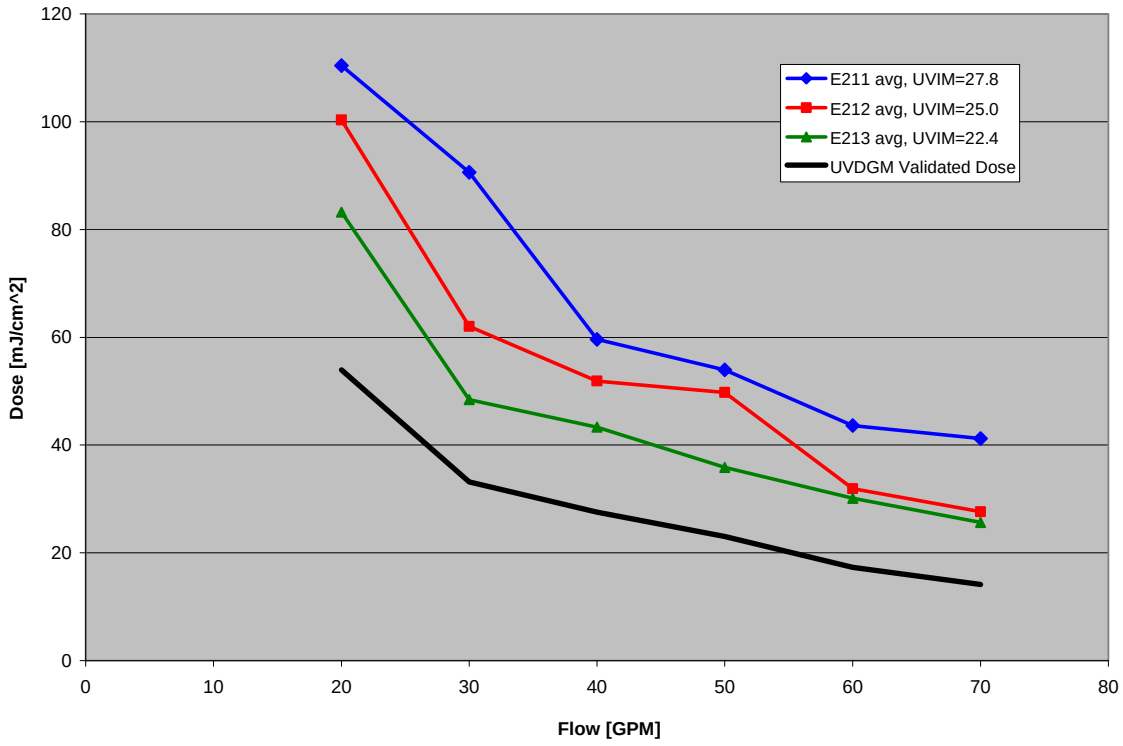

Figure 8. Reduction equivalent dose (RED) for NeoTech D222 with 95% UVT water.

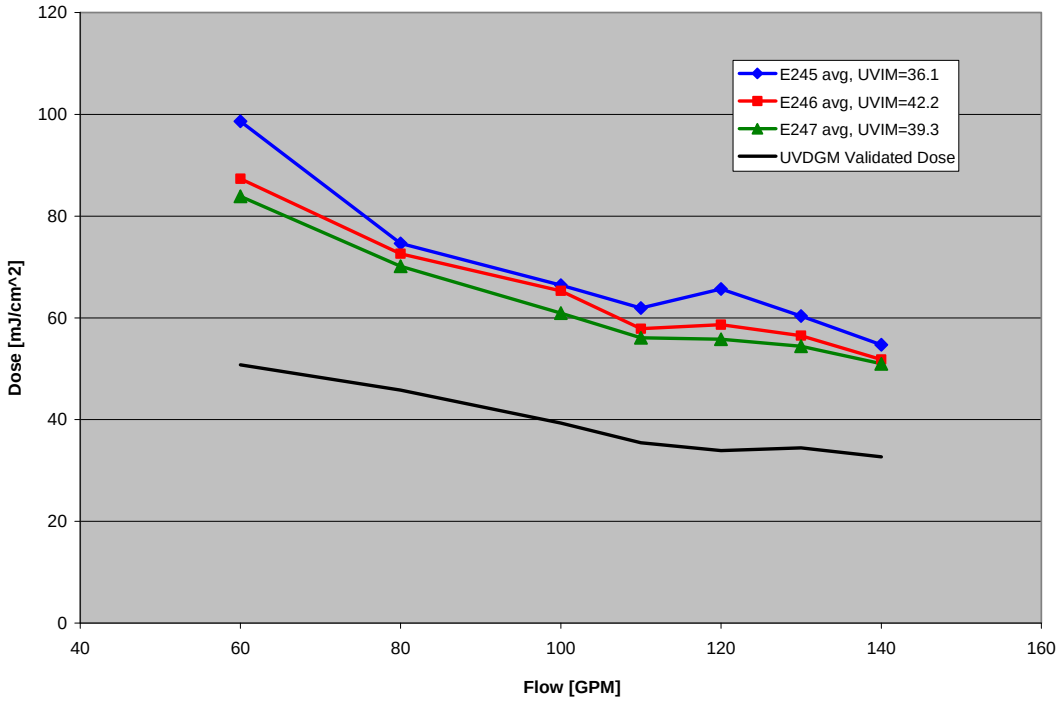

Figure 9. Reduction equivalent dose (RED) for NeoTech D238 with 95% UVT water.

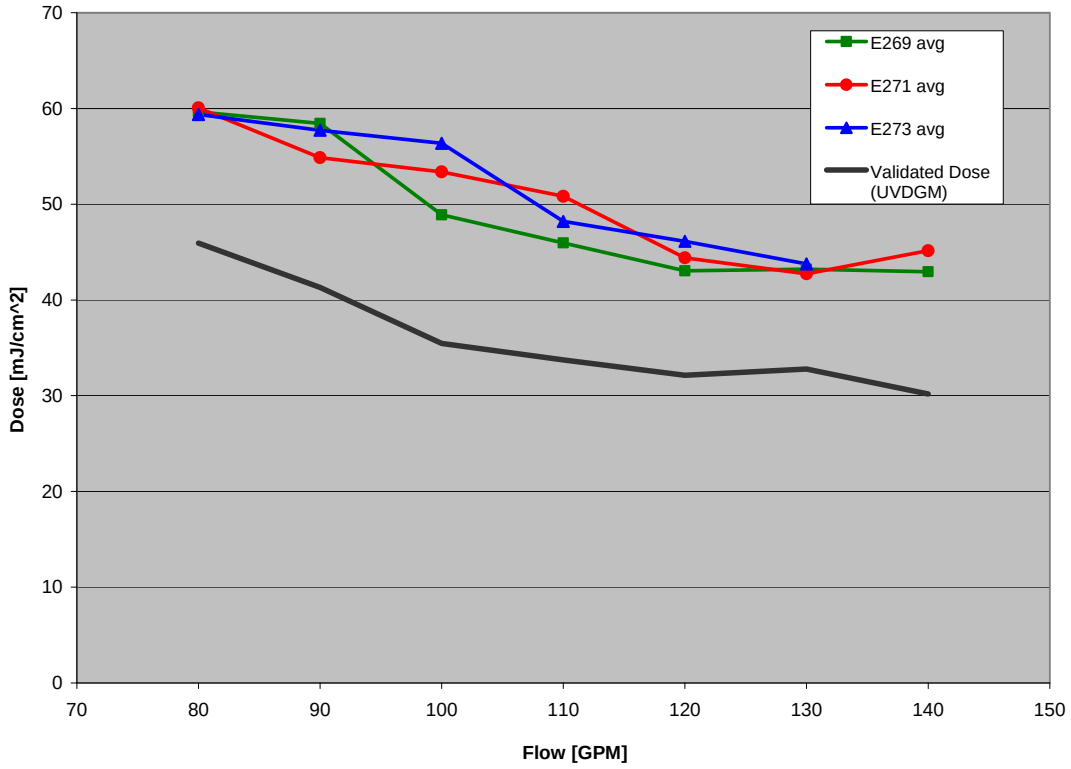

Figure 10. Reduction equivalent dose (RED) for NeoTech D322 with 95% UVT water.

4.3 Third Party Test Data for NeoTech Aqua Systems

As mentioned in the introduction, third party data has been collected to confirm the results obtained with our in-house experiments. This requires that the tests at both places be performed under the same conditions to the extent possible for them to be used as a basis for comparison. Figure 11 shows a comparison of in-house data taken with a NeoTech Aqua D338 up to the maximum test system capability of 160 GPM for that unit, compared with Data taken at the National Sanitary Foundation's (NSF) test facility in Ann Arbor, Michigan. This facility is capable of testing to flow rates of up to 2000 GPM. For the test data presented in Figure 11, the nominal readings for the UV Intensity Monitor on the NeoTech data are in the range of 9-11 mW/cm^2, and the measured intensity in the NSF tests is 13 mW/cm^2. In order to compare these data, the NeoTech data have been linearly scaled to correspond to the same UV intensity reading as was measured at NSF.

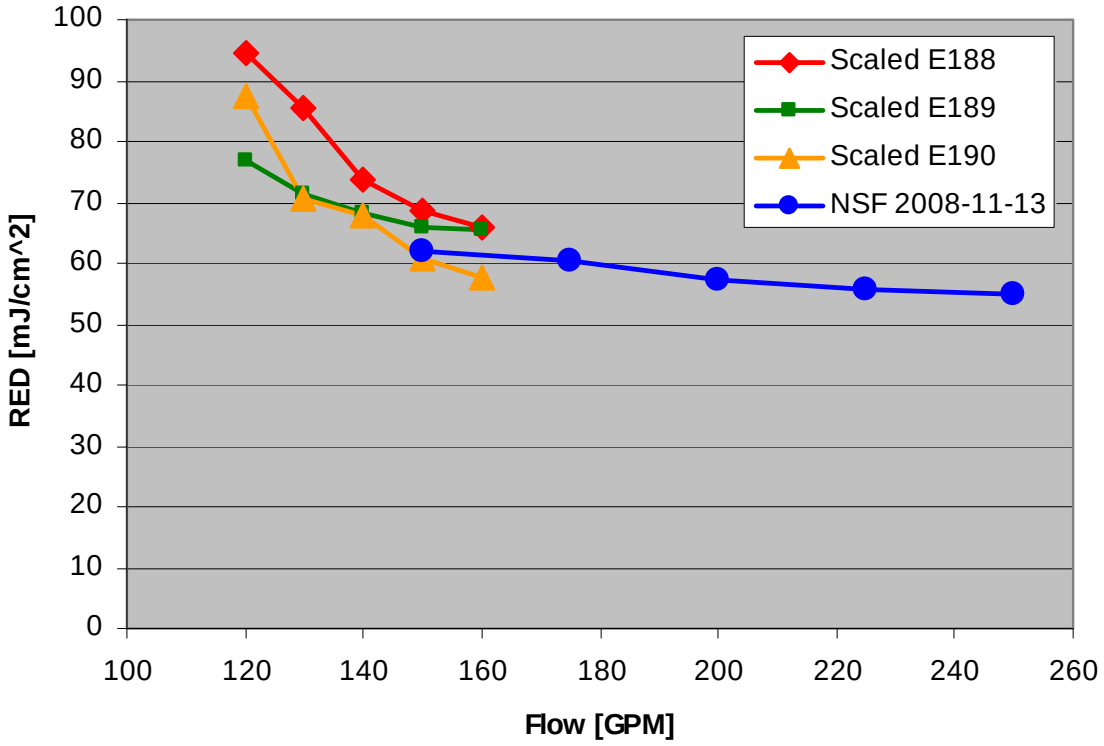

Figure 11. Reduction equivalent dose (RED) for NeoTech D338 with ~95% UVT water. The data listed as scaled are adjusted to provide a proper comparison of the two data sets as described in the text.

After this scaling, the graph shows very good agreement of the two data sets at the flow rates where they overlap. This agreement, coupled with the use of widely accepted protocols and NIST-traceable diagnostics, provides a strong basis for the validity of both testing procedures.

The data collected by NSF for the NeoTech Aqua D438 is shown in Figure 12 below, along with the calculated validated dose based on those tests.

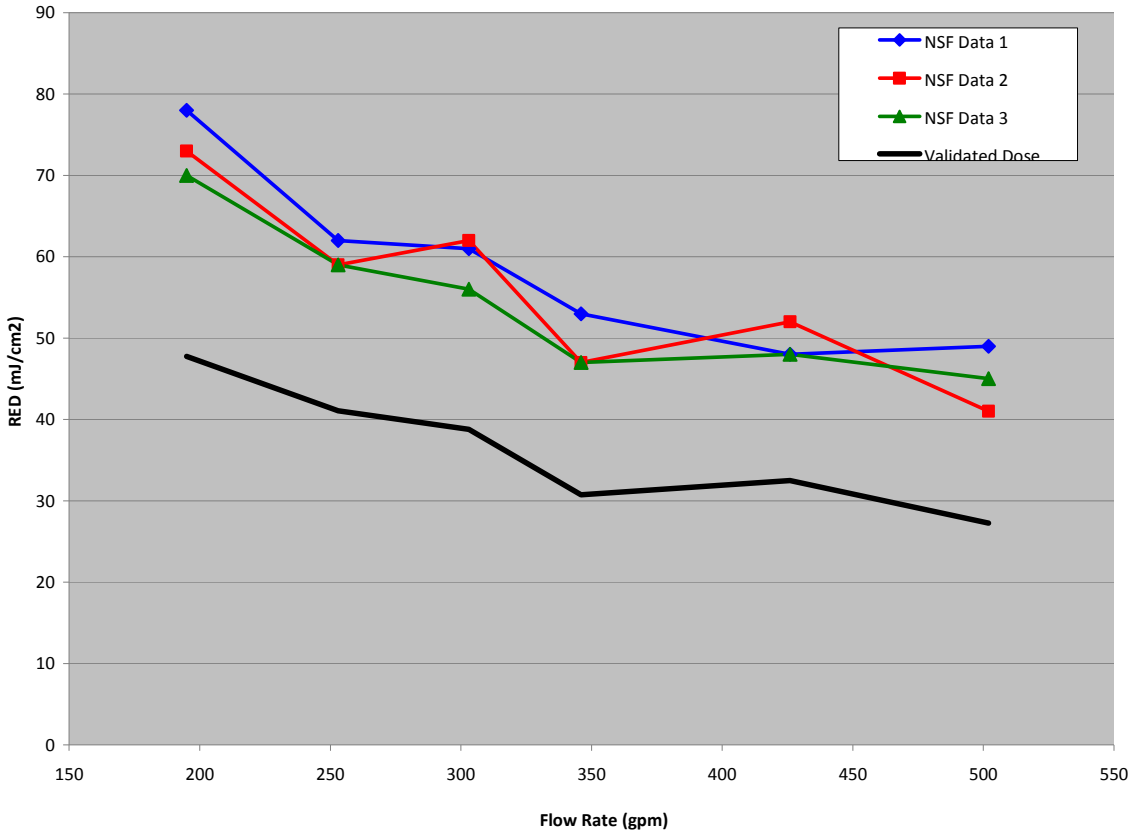

Figure 12. Reduction equivalent dose (RED) for NeoTech D438 with 95% UVT water. All data taken at NSF

Figure 12 shows that the validated dose as defined by the EPA UV Disinfection Guidance Manual for the NeoTech D438 is 461 GPM at 95% UVT. As discussed before, the validated dose is a statistically-defined worst case performance, so the expected dose for a given flow rate and UVT will almost always be significantly higher than the validated dose

5. Conclusions

The efficacy testing described in this document follows a protocol established by thought leaders in the industry. Minor deviations from this protocol were made to improve performance, accuracy and throughput. A standard challenge microorganism was utilized, and the data collected correlates well with published data from a number of other sources using the same strain of that microorganism.

In-house results for all of the tests performed were repeatable to a high degree of accuracy using different generations of microorganisms on at least three different test dates. This indicates that the reliability of the testing done is sufficient to characterize the

performance of the UV reactors tested. The validated dose generated by the reactors exceeds their ratings for all allowable operating conditions, indicating that, if operated properly, they will always meet or exceed their stated specifications.

References

[1] U.S. Environmental Protection Agency, Office of Water (4601), Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule, EPA 815-R-06-007, November 2006.

[2] American Society for Microbiology, "Anthrax Spores May Survive Water Treatment," Science Daily 26 February 2006. <http://www.sciencedaily.com/releases/2006/02/060226115234.htm>