Not All Water Is Created Equal: The Effects of Water Characteristics on MS2 Stability and Dose Response

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Ultraviolet (UV) reactors typically have their performance claims verified using a bioassay which involves running water spiked with a surrogate microbe, such as MS2 bacteriophage, through the system under a variety of flow, UVT and system power conditions, where the reduction of the surrogate is measured. Water characteristics are one condition that is often overlooked but can drastically impact the results obtained during the validation. For large UV systems, validations are typically performed at dedicated facilities in North America and Europe where data has been collected over many years of testing. This has shown that the dose response results obtained from these facilities is very reproducible and predictable for the surrogates typically used. Issues with dose response curves and apparent log reductions from bioassay samples begin to arise with the use of novel validation surrogates in new water sources, such as validation work of small scale systems using a new water source or alternative validation surrogates at test sites, such as bacteriophage (UVDGM) validations in Europe or *Bacillus* (DVGW/ÖNORM) validations in North America.

These issues had not appeared in the past, as validations had been confined to existing dedicated test facilities with novel surrogate usage; but validation in these dedicated facilities is often a barrier for testing to multiple international standards, so interest was piqued in the use of alternative validation surrogates at existing facilities. Testing of smaller or novel systems, often from smaller, or start-up companies is also often cost prohibitive or impractical at these dedicated facilities which has led to an interest in validations occurring at manufacturers facilities, at public/private/academic laboratories or even at installation locations.

When testing UV reactors to international standards all standards make a statement regarding the water that must be used. The NWRI requires site specific or finished drinking water for drinking water validations and granular media filtered reclaimed water with less than 1 ntu for reuse water validations (NWRI 2012). The NSF specifies the pH, turbidity, and total dissolved solids of the test water (NSF 2016), and the DVGW specifies the Fe/Mn content, disinfectant residual, turbidity, and background microbial content (DVGW 2006). The only standard that makes specific reference to testing the suitability of the water with the validation microbe and not on specific water quality parameters is the UVDGM (2006). Here, the UVDGM suggests the use of reactor controls, reactor blanks, trip controls, method blanks, and stability samples. These quality control parameters are specifically defined for reactor controls, reactor blanks, trip controls, method blanks, but for stability samples the requirements are less specific, saying that different water conditions should be tested, such as high and low UVT, and that the dose response over time must be verified. In the past this requirement had been achieved by determining the concentration and measured log reductions of samples day over day, including for collimated beam samples, and there has not been a problem with the results we have seen. What we show here is that it should also include a confirmation that the actual dose response, as determined by a collimated beam test, falls within an expected range for these same high and low UVT water conditions.

Using the statement from the UVDGM that states for stability testing there be no die off over the course testing from sample collection to the completion of the laboratory assay procedures (UVDGM) does capture that distilled water is inappropriate for validation testing, as can be seen in Figure 1, but there are

several other water types that appear to be a good fit for validation that we can show using other methods to be an incorrect conclusion.

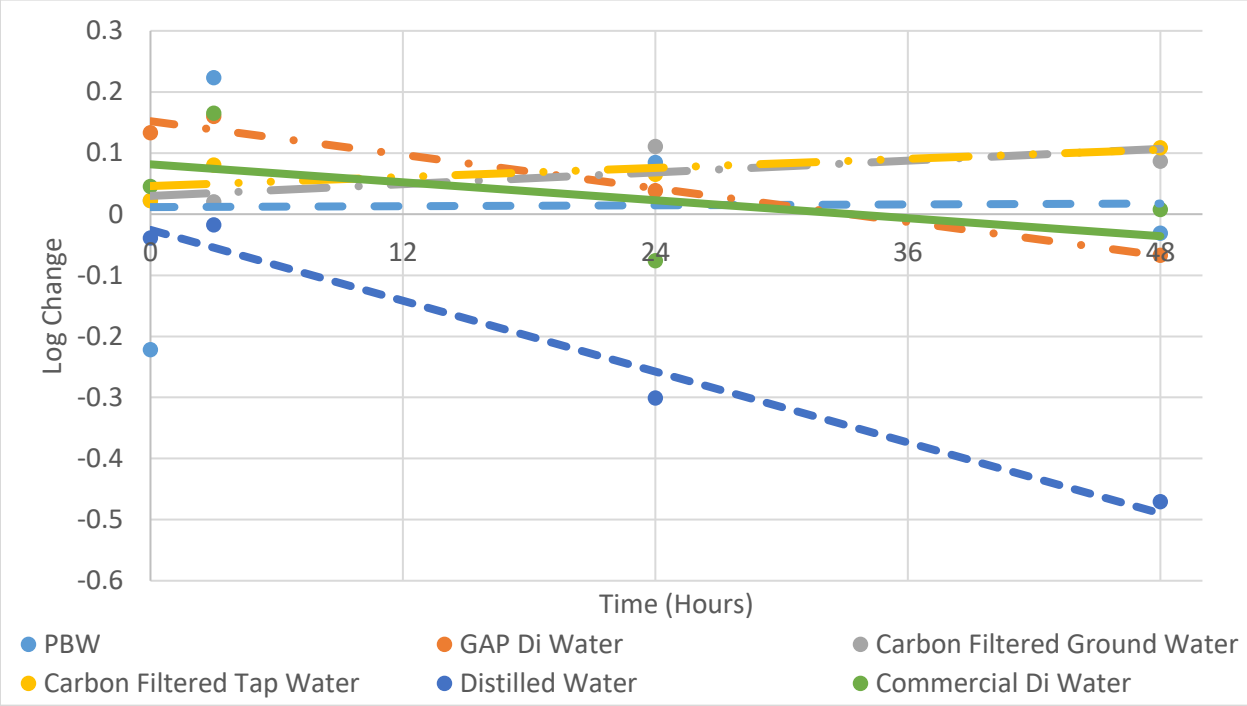

Table 1: The change in the log concentration of MS2 over time in different water types.

Another component of the UVDGM definition of stability sample testing is that the UV dose response remain stable over the course of laboratory analysis. What is not mentioned as part of this definition is that the dose response must also sit within an expected range for the surrogate used, and this is what was found to be the most important aspect in determining whether a water type is appropriate for validation testing. This expected dose response can be, preferably, determined using a historical data set for a microbe to calculate confidence bounds (Verhoeven et al. 2016), from recognized published data, or, if no other source exists, from a quality control curve produced by a laboratory in phosphate buffered water. Recognized published data should not include the bounds published in the UVDGM as the range is too wide and they were not intended to be quality control limits. The bounds published in the NWRI manual are a better alternative as they are defined as quality control limits, but large numbers of historical data will allow for the shrinking of these bounds even further, providing the most confidence that the curve produced is accurate and the water used for testing is appropriate.

To determine the UV dose response of a microbe in the test water, the only way to do this is using a collimated beam test. This procedure is well defined in literature, and several laboratories and manufacturers have the equipment required to perform this testing. This collimated beam testing should be done, at minimum, before using a new water source is used or a new UVT modifier/water combination is used with a new surrogate microbe. Ideally this would also be done throughout validation testing to ensure that the water and UVT modifier/water combination is not changing throughout the testing. By performing a collimated beam test on the same waters shown in Table 1, we can see in Table 2 that other waters, GAP de-ionized water and commercially available de-ionized water, are also inappropriate for performing validation testing using MS2. Simply accepting that the water will produce the same dose

response with each re-irradiation is also an incorrect assumption as can be seen in Table 3. Here the same water types used for the irradiations in Table 2 were re-irradiated 3, 24, and 48 hours later and the curves produced did not match the first irradiation. This shows that simply accepting the dose response results as correct for a curve that does not fit inside the expected bounds is not an acceptable approach to validation, as the measured fluence or dose will not be the same with repeated exposures or comparisons to a collimated beam curve. This would mean that the log reduction measured from a flow through experiment, may not necessarily compare accurately to a collimated beam test, therefore having the collimated beam dose response fitting as expected is essential to proving a validation has been successfully performed. What we can also observe from the collimated beam results outlined in Table 2 and Table 3 is that there is a large amount of scatter in the curves for water types that fall outside the GAP upper and lower bounds. This further shows that there is a high level of uncertainty for the log reductions calculated both through a collimated beam test and from flow though reactor validation samples for waters that are inappropriate for validation testing.

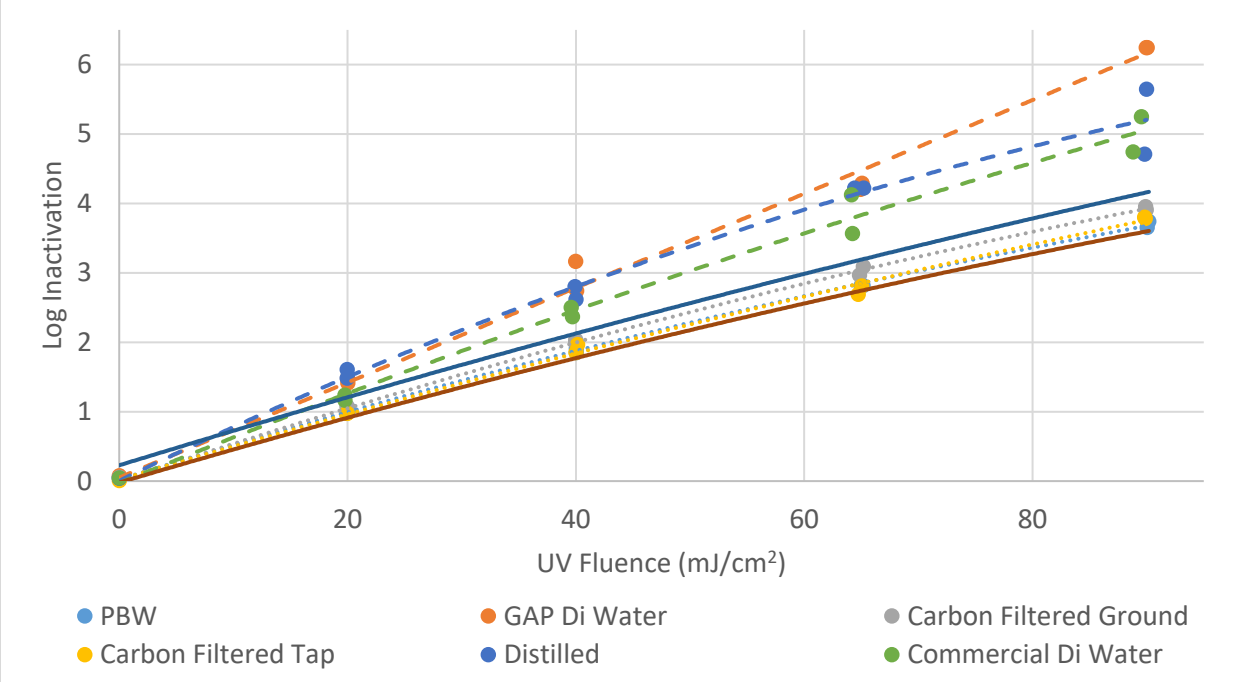

Table 2: Dose response curves generated for several different water types immediately after addition of the MS2 to the water. Solid lines represent the internal GAP limits for MS2 collimated beam tests.

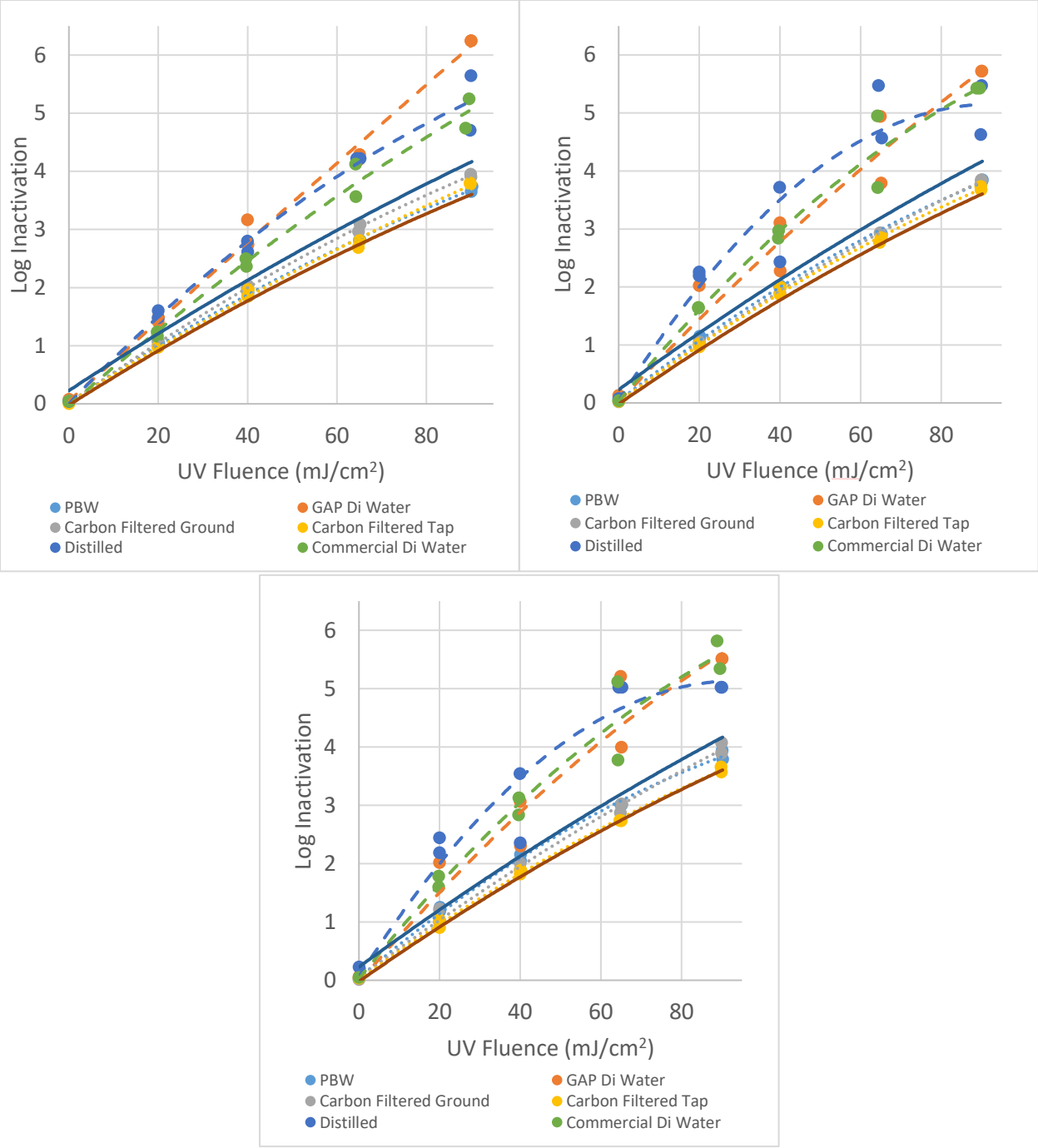

Table 3: Dose response curves generated for several different water types 3 hours (top left), 24 hours (top right), and 48 hours (bottom) after addition of the MS2 to the water. Solid lines represent the internal GAP limits for MS2 collimated beam tests.

Using a collimated beam test before and during testing is essential to determine the appropriateness of water types. The water types that appeared to be inappropriate for validation testing above would likely only be used for the validation of small scale reactors with small flow rates, but there are instances in large scale validations where this issue can also arise. We have seen that water from certain ground water

sources can appear appropriate for validation testing when MS2 is added directly to the water and a collimated beam performed, but once a UVT modifier is added, in this case LSA, then the resulting curve falls well outside of the acceptable range, as can be seen in Table 4. This effect does not appear with all ground water sources, which proves how important collimated beam testing is in determining the validity of a validation test. With Table 4 we can also see that not all UVT modifiers cause the dose response curve to fall outside of bounds, as Super Hume does not affect the curve when added to the same water, and the issue does not lie with LSA, as the curve produced when LSA is added to phosphate buffered water is within bounds. There is some combination of the water and LSA that is causing the curves to produce unexpected results.

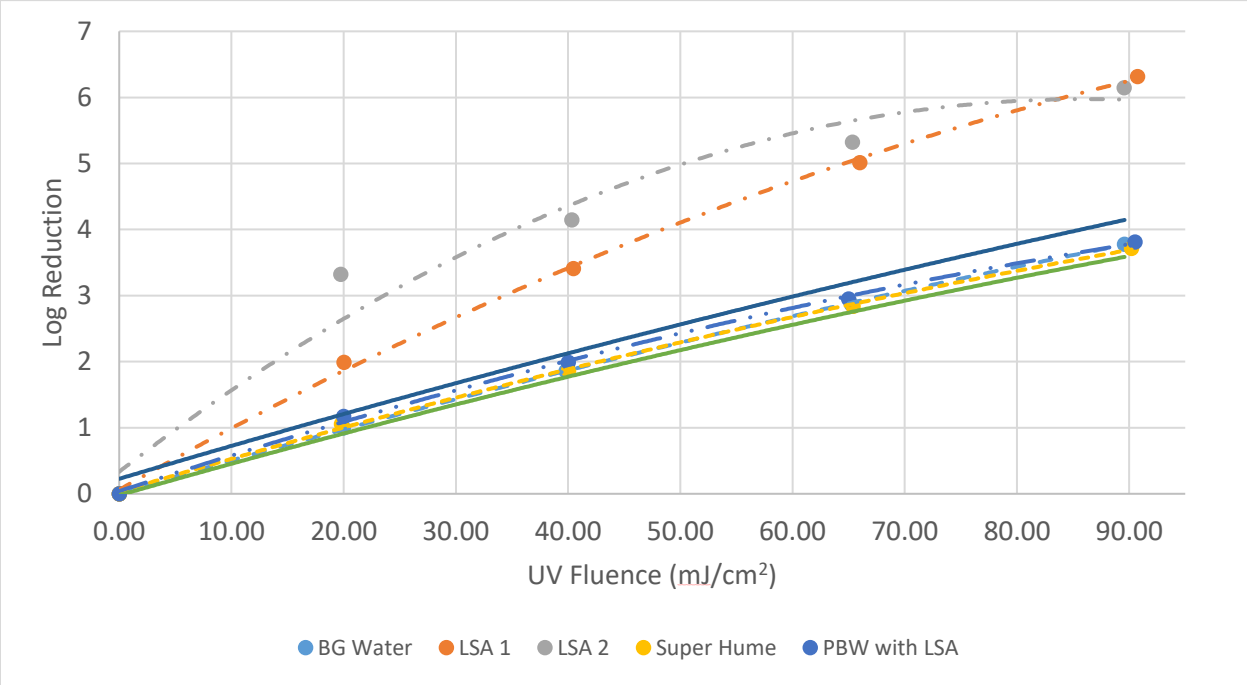

Table 4: Groundwater from one source with different UVT modifiers added, along with phosphate buffered lab water with LSA as a control.

In conclusion, there are many water types and combinations that can absolutely influence a validation. We do not yet know the exact causes of these deviations, but there are steps that can be taken to ensure the results produced during a validation are accurate. This includes performing a collimated beam and day over day stability on all combinations of water before starting validation using a new water source, surrogate microbe, or UVT modifier in a water. Collimated beam testing throughout validation is also an important, as any changes in the water effect on the surrogate cannot be monitored if this step is omitted. Also, simply using a calibration curve produced for the surrogate by a laboratory using phosphate buffered water, for quality control purposes, is not acceptable, as this would not consider the effects of the validation water itself. When considering the water combinations that must be tested when determining the appropriateness of water, all surrogates, water types, and UVT extremes should be considered, as all these combinations may individually affect the validation results. If dose response curves fall within the expected bounds, there is a high level of confidence that the results produced from a flow through validation are also accurate and unaffected by the water. Also, it isn't acceptable to use the dose response

curve that falls outside of the established limits, as we have shown that this inactivation may not be repeatable and can vary greatly over repeat exposures.

Currently we are investigating to see if there are specific water quality parameters that can be measured to determine whether a water is appropriate for validation testing, and if there is any way to recover MS2 that appears to be inactivated beyond what UV itself causes. Since we are still working on this, the only way to avoid the potential risks is to follow the steps outlined above. Collimated beam testing does add to the cost of a validation, especially on small scale validations, but are necessary, as we must be able to attribute all apparent log inactivation of MS2 to the UV, and not the water. MS2 may not be the only surrogate microbe affected by the water conditions, so testing all microbes used during validation is important.

References

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