

# UV Action Spectra of Pathogens and Surrogates

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The use of bioassays to determine reduction equivalent doses delivered by UV reactors is recognized as an effective measure of UV disinfection systems. Low pressure (LP) UV delivered in controlled collimated beam systems provides dose response curves which are then compared against reactor performance to quantify the delivered dose. It is recognized that the appropriateness of monochromatic LP dose curves for validating polychromatic medium pressure depends on knowledge of the action spectra of the bioassay surrogate and the target pathogen. Further complicating the relationship between reduction equivalent doses (RED) generated during validation and effective dose delivered by a medium pressure UV system at an installation is the absorbance spectrum of the validation water versus that at the utility. Other factors that affect the dependability of bioassay results for medium pressure systems include quartz sleeve absorbance spectra and lamp spectral output. These latter factors can be resolved or corrected for, but not without knowledge of the action spectra of the microorganisms in question.

Existing characterization of the action spectra of pathogens and bioassay surrogates is limited, and ranges from two published (Rauth, 1965; Mamane-Gravetz, et al. 2005) and three unpublished studies on the commonly used surrogate, coliphage MS2, to one published study on *Cryptosporidium* (Linden et al., 2001) and adenovirus (Malley et al, 2004), to no information on several surrogates. Further, the methodologies used to irradiate microbes with distinct wavelengths have varied across studies. Data reported as representative of the relative inactivations of given wavelengths may actually represent the inactivations achieved by a range of wavelengths produced by a polychromatic lamp filtered by wide-width bandpass filters.

The objective of this study was to compare the action spectra of the pathogens targeted by UV disinfection of drinking water with those of the microbial surrogates used in bioassay validations (see Table 1). Pathogens studied were *Giardia*, *Cryptosporidium*, and adenovirus Type 2. Surrogates tested include the coliphages MS2, T1UV, Q beta, and spores of *Bacillus pumilus*. While

medium pressure UV and bandpass filters would be initially used with the surrogates, the study was designed to definitively measure the action spectrum of each microorganism when irradiated with monochromatic UV as delivered by a tunable laser source.

**Table I:** Microorganisms evaluated in study

Microorganism	Source	Notes
MS2	ATCC (15597-B1)	Host: <i>E. coli</i> 15597-1
T7m	ATCC (11303 B38)	Host: <i>E. coli</i> B
Q beta	ATCC (23631-B1)	Host: <i>E. coli</i> 23631
T1UV	GAP Microenvironmental, Conrad Odegaard; (available from Laval University/HER#468)	Host: <i>E. coli</i> CN13
<i>Bacillus pumilus</i> ASFUVR	Paul Rochelle, MWD	0.1 mol/m <sup>3</sup> (mM) MnSO <sub>4</sub> added to media during propagation
Adenovirus 2	ATCC (VR-846)	Cell culture; A549 cell line; MPN enumeration
<i>Cryptosporidium parvum</i> (Iowa strain)	Waterborne	Cell culture infectivity
<i>Giardia intestinalis</i>	USEPA	Gerbil infectivity assay

## Irradiation methods

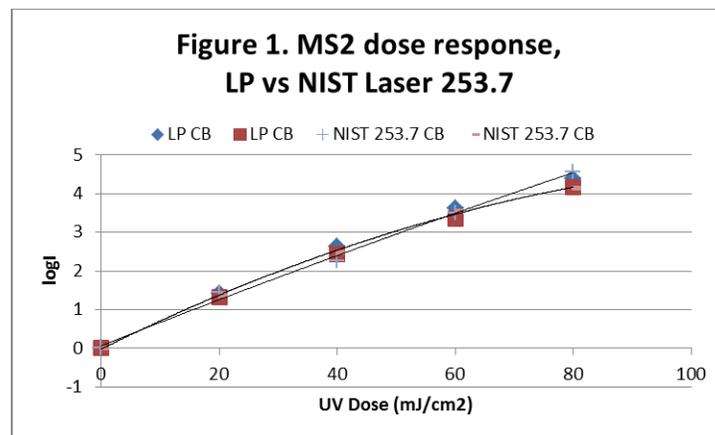
All exposure times were calculated using the methodologies described by Bolton and Linden 2003. Four light sources were used in this study. Low pressure UV was produced by a 15 W Atlantic Ultraviolet lamp, housed in a collimated beam (CB) apparatus, and was used to generate dose response curves at nominal 254 nm. The same CB unit also accepted a 1 kW medium pressure Rayox lamp, which was utilized for the initial series of wavelength dose response curves developed by passing the UV polychromatic light through a series of bandpass filters. Spectral output through each filter was determined using an Ocean Optics spectral radiometer. Peak widths were generally 5 nm to 10 nm at 50% of peak height. Because output at 200 nm from the medium pressure lamp was quite low, a deuterium 2 lamp was used with the 200 nm bandpass filter for the 200 nm exposures. The *B. pumilus* action spectrum was developed with the bandpass filters only.

For irradiations of precise wavelengths, a tunable laser system developed and operated by the National Institute of Standards and Technology (NIST) was utilized. This system consisted of an Ekspla tunable pulsed laser at 1000 Hz repetition rate, which produces precise wavelength laser beams by means of doubling and tripling crystals. On exiting the laser, the beam was directed by a series of dielectric or aluminum mirrors to a prism and slit, depending on wavelength. These were installed to eliminate visible wavelengths at twice the target wavelength. In a final step, the beam is directed to a diffuser which spreads the narrow laser out sufficiently to uniformly irradiate the test microbes in a petri dish of 36 mm diameter. The irradiance so produced has a narrow band peak irradiance of approximately 2 nm width at 50% of peak. Measurements of the distribution over the petri dish surface, made each time the wavelength was changed,

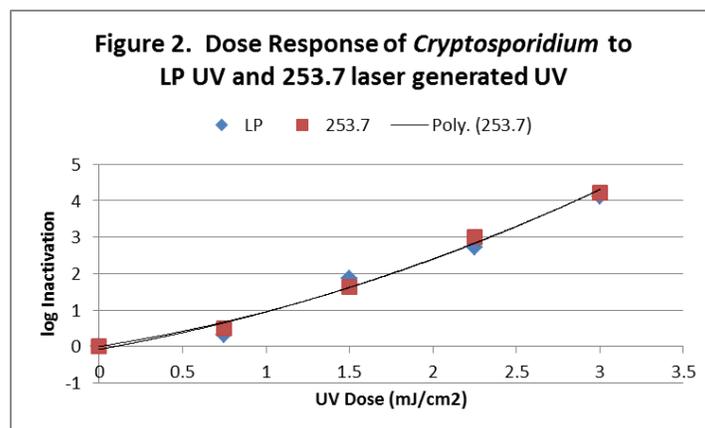
demonstrated a petri factor within the range of 0.94 to 1.05. The lowest wavelength that the laser system could produce was 210 nm. Irradiances in the range of 25  $\mu\text{W}/\text{cm}^2$  to 180  $\mu\text{W}/\text{cm}^2$  were achieved by the laser and optics, depending on wavelength. As *Giardia* and *Cryptosporidium* require very low doses for 1 log to 4 log inactivation, neutral density filters were used to diminish irradiation to the point that exposures of at least 30 s were required. For all microorganisms, a range of doses expected to achieve 1 log to at least 3 log inactivation by the target wavelength were selected.

## Results

The immediate focus of this study has been on the relative action spectra of coliphage MS2 and *Cryptosporidium*, and the action spectrum of adenovirus, as generated by the tunable laser UV source. As a first check of the laser system, the dose responses of *Cryptosporidium* and MS2 with the wavelength set at 253.7 nm was compared to that generated by a low pressure UV (253.7 nm) lamp. The results for each light source and microbe are given in Figures 1 and 2, demonstrating excellent comparability of the laser with low pressure mercury UV.



**Figure I:** MS2 dose response, LP vs NIST Laser 253.7



**Figure II:** Dose Response of *Cryptosporidium* to LP UV and 253.7 laser generated UV

Action spectra are being developed for each microorganism tested, by normalizing the inactivation required for 2 log inactivation by each wavelength to that attained by the laser at 253.7 nm. Preliminary analysis indicates that *Cryptosporidium* is more sensitive to wavelengths below 240 nm than are the coliphage surrogates, and that MS2 is the most sensitive phage in that range. Adenovirus Type 2, however, is much more sensitive than MS2. The microbes tested were more similar at wavelengths above 253.7 nm, with *Cryptosporidium*, in the midst of the phage curves, quite similar to MS2.

As these action spectra are being produced by very narrow peak irradiances, they offer higher confidence in our understanding of the relative impact of distinct wavelengths on bioassay surrogates and target pathogens in drinking water. As such, they will aid manufacturers, regulators, water utilities, and design engineers in providing properly designed and monitored UV systems to efficiently protect public health. Final action spectra will be presented at the WQTC conference and available from the Water Research Foundation in the final report for the project coming out in 2014.

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Atlantium Ultraviolet, Har Tuv Israel

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ETS UV Technology, Beaver Dam WI, USA

Trojan Technologies, London Ontario, Canada

Xylem, Herford, Germany

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The mention of certain commercial products in this paper is for information purposes only and does not constitute an endorsement of the product by the authors or their institutions.

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