Poster # AAR-712

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RNase A Activity as a Predictive Tool for Log_{10} Reduction of Bacterial and Fungal Spores on Surfaces

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Abstract:

Certain clinically relevant organisms, such as *Clostridium difficile* and *Aspergillus brasiliensis*, form spores that are resistant to UV inactivation. Indeed, Log_{10} reduction in response to UV exposure is dependent not only on dose, but also on irradiance and wavelength(s) of the light.[1,2] Experiments designed to achieve high Log_{10} reduction in CFU are time consuming, iterative, costly, require production of large numbers of spores, and must be conducted with proper biological containment. In this study we investigated the use of RNase A as a surrogate for estimating the effectiveness of high-intensity UV LED in reducing colony forming units of bacterial and fungal spores on surfaces, prior to culture studies.

One microliter of dried RNase A (Worthington, 2 U/µl, 0.2 U/µl, 0.1 U/µl, 0.05 U/µl, 0.02 U/µl) was exposed to UV LED under conditions matching those used for *C. difficile* and *A.brasilliensis* spores to achieve >5 Log_{10} reduction and >4 Log_{10} reduction, respectively. RNase A activity of the resuspended samples was assayed using the RNase Alert substrate and buffer (IDT). Fluorescence was detected on a GeminiXS microplate fluorimeter (Molecular Devices). Reactions were allowed to continue for one hour.

C. difficile spores (~3 x10⁶ cfu/target) were exposed to 275 nm UV LED at three different irradiance levels (122.9 mW/cm², 245.7 mW/cm², and 491.5 mW/cm²). *A. brasiliensis* spores (~4.4 x10⁵ cfu/target) were exposed to combined 275 nm (492.1 mW/cm²) + 365 nm UV LED at four different irradiances (0 mW/cm², 180.1 mW/cm², 360.2 mW/cm², and 720.3 mW/cm²). Surviving CFUs were counted and Log_{10} reductions calculated.

Distance from the source to the target was 25mm in all cases.

Under the conditions that resulted in a >5 Log_{10} reduction in *C. difficile* spores and a >4 Log_{10} reduction in *A. brasilliensis* spores, the 0.02 U/µl RNase A sample tracked the negative control. The 0.1 U/µl sample was consistently above negative control. The differing concentrations of RNase A show distinct levels of inactivation in response to UV LED exposure conditions, just as the Log_{10} reduction of the spores varies in response to corresponding exposure conditions.

The RNase A inactivation assay can be used to estimate *C. difficile* and *A. brasillieinsis* spore response to UV LED exposure. This assay may be used to narrow the conditions needed for experiments on these organisms. It is likely that this same assay method could be used to estimate the UV LED response of other organisms.

Introduction:

Certain clinically relevant organisms, such as *Clostridium difficile* (*Clostridioides difficile*) and *Aspergillus brasiliensis*, form spores that are resistant to UV inactivation. Indeed, Log_{10} reduction in response to UV exposure is dependent not only on dose, but also on irradiance and wavelength(s) of the light.[1] A similar observation was made when testing methods for RNase A inactivation. RNase A is an exceptionally stable enzyme that can retain much of its activity even after autoclave treatment. We found that the enzyme inactivation could be controlled by irradiance, wavelength, and dose delivered to an enzyme contaminated surface.[2,3] Experiments designed to achieve high Log_{10} reduction in CFU of spore forming microbes are time consuming, iterative, costly, require production of large numbers of spores, and must be conducted with proper biological containment. For *A. brasiliensis* and *C. difficile* the containment levels are BSL-1 and BSL-2, respectively. In this study we investigated the use of RNase A as a surrogate for estimating the effectiveness of high-intensity UV LED in reducing colony forming units of bacterial and fungal spores on surfaces, prior to culture studies.

Methods:

RNase A experiments: One microliter of dried RNase A (Worthington, 2 U/µl, 0.2 U/µl, 0.1 U/µl, 0.05 U/µl, 0.02 U/µl) was exposed to UV LED under conditions matching those used for *C. difficile* and *A.brasilliensis* spores to achieve >5 Log_{10} reduction and >4 Log_{10} reduction, respectively. Enzyme contaminated surfaces were exposed at a distance of 25 mm from the light source. RNase A activity of the resuspended samples was assayed using the RNase Alert substrate and buffer (IDT). Fluorescence was detected on a GeminiXS microplate fluorimeter (Molecular Devices). Reactions were allowed to continue for one hour.

Microbial Experiments: *C. difficile* spores (~3 x10⁶ cfu/target) were exposed to 275 nm UV LED at three different irradiance levels (122.9 mW/cm², 245.7 mW/cm², and 491.5 mW/cm²). *A. brasiliensis* spores (~4.4 x10⁵ cfu/target) were exposed to combined 275 nm (492.1 mW/cm²) + 365 nm UV LED at four different irradiances (0 mW/cm², 180.1 mW/cm², 360.2 mW/cm², and 720.3 mW/cm²). Microbe contaminated surfaces (1"x1" targets) were exposed at a distance of 25 mm from the light source. Surviving CFUs were counted and Log_{10} reductions calculated.

Light Source: A hi-intensity air-cooled dual wavelength LED lamp was computer controlled for independent wavelength control, exposure time, and drive current needed to supply controlled doses of each UV wavelength. For conditions where samples exposed to both 275 nm and 365 nm UV, exposure was simultaneous.

Analysis: Log reduction of CFU was calculated from four independent platings of serial dilutions of each of three independent repetitions of each UV exposure condition.

Results:

The differing concentrations of RNase A show distinct levels of inactivation in response to UV exposure conditions, just as the Log_{10} reduction of the spores varies in response to correspLED onding exposure conditions. As seen in Figure 1, 275 nm UV exposure of *A. brasiliensis* spores plateaus at 1.65 Log_{10} reduction. Addition of 365 nm UV in combination with the 275 nm UV plateaus at a 4.95 Log_{10} reduction.

C. difficile spores are more sensitive to 278 nm UV than the *A. brasiliensis* spores. Exposure of *C. difficile* spores to 275 nm UV alone plateaus at a 5.79 Log_{10} reduction. Addition of 365 nm UV in combination with the 275 nm UV results in maximum 5.79 Log_{10} reduction under all tested conditions. For these assays a 5.79 Log_{10} reduction corresponds to the maximum statistical power of the assay. Note that the actual reduction may be higher when the study is repeated with a higher statistical power.

Under the conditions that resulted in a >5 Log_{10} reduction in *C. difficile* spores and a >4 Log_{10} reduction in *A. brasilliensis* spores, the 0.02 U/µl RNase A sample tracked the negative control. The 0.1 U/µl sample was consistently above negative control. Finer discrimination may be possible based on the results for *A. brasiliensis* suggesting an upper limit of 0.05 U/µl RNase A for the combination 275 nm + 365 nm exposures.

Conclusion:

- The RNase A inactivation assay can be used to estimate the response of *C. difficile* and *A. brasilliensis* spores on a surface to UV LED exposure.
- This RNase A inactivation assay may be used to narrow the conditions needed for experiments on these organisms.
- It is likely that this same assay method could be used to estimate the UV LED response of other organisms.
- Once matched to the organism of interest in a lab or manufacturing area this type of assay may be used to monitor surface contamination on a frequent basis. Potentially providing early warning of contamination for organisms that require multiple days to culture.

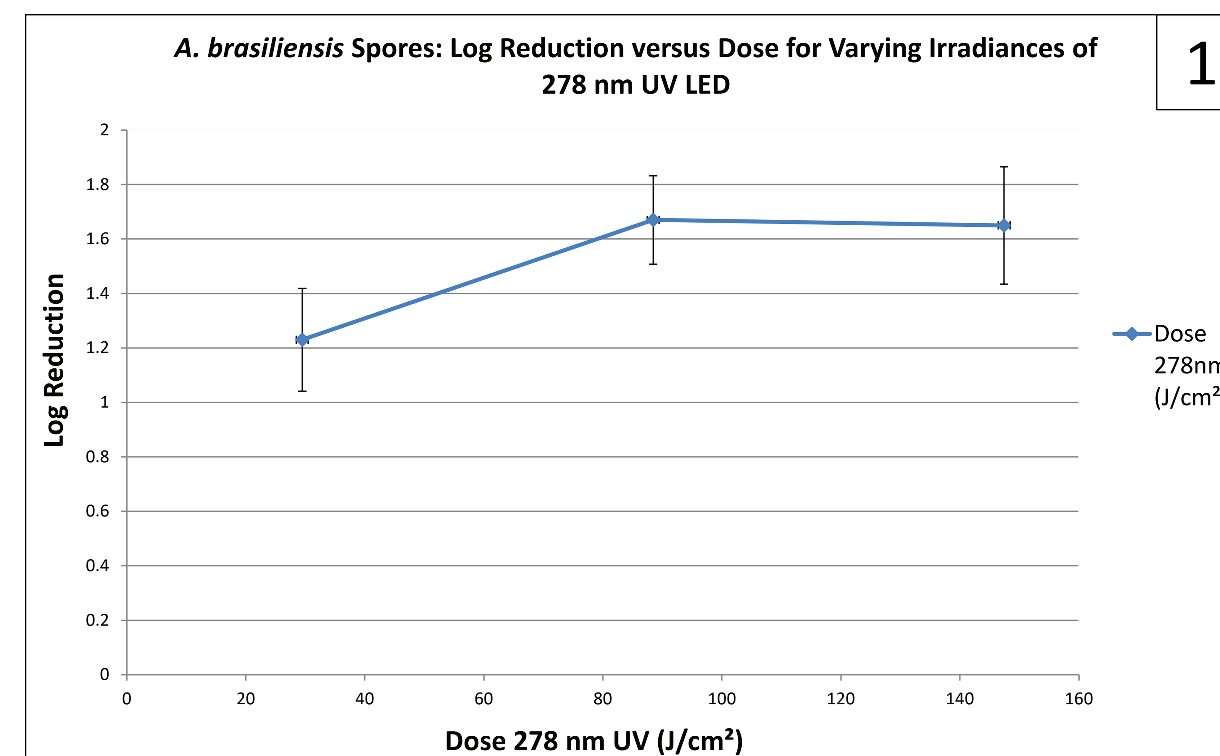

Figure 1: Log reduction of *A. brasiliensis* CFU recovered from surfaces after hi-intensity 278 nm UV exposure (491.5 mW/cm²). Spores were exposed to 275 nm UV LED for 60, 180 and 300 seconds. The resulting doses were 29.5, 88.5, and 147 J/cm². Log reduction plateaued at 1.65 Log_{10}.

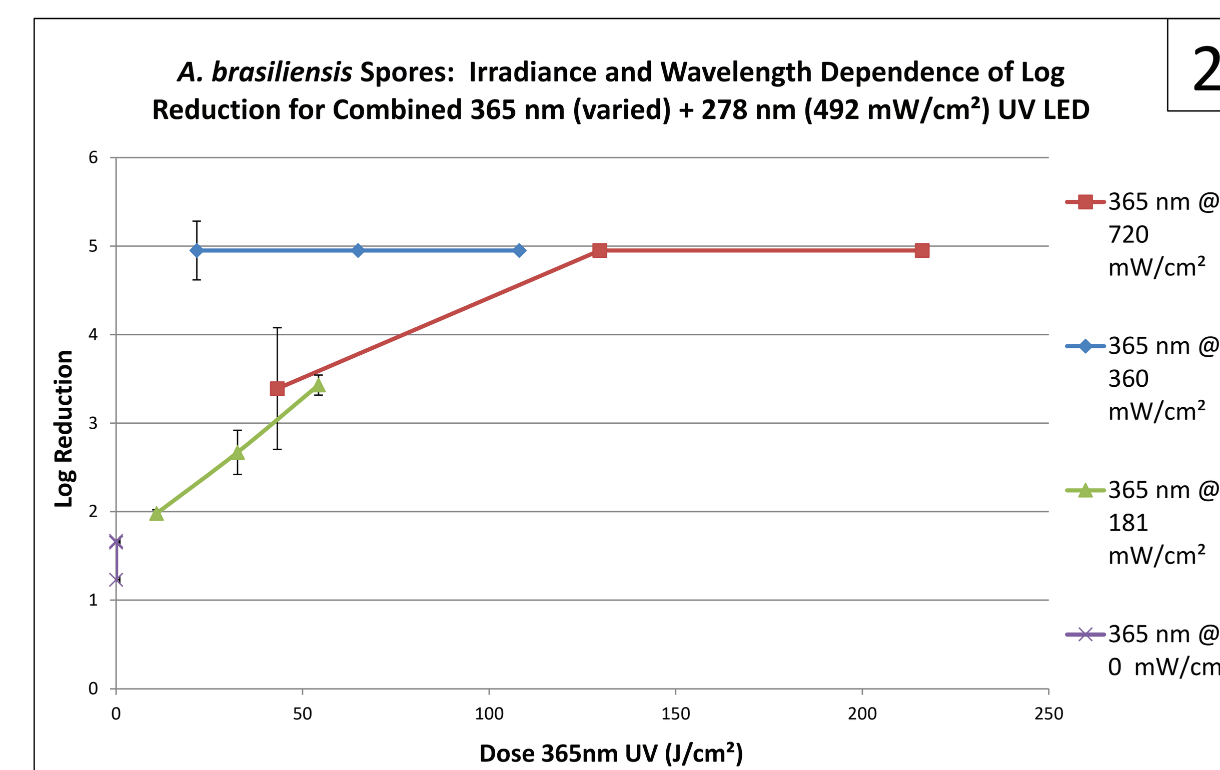

Figure 2: Log reduction of *A. brasiliensis* CFU recovered from surfaces after hi-intensity combined 278 nm + 365 nm UV exposure. Spores were exposed to 275 nm (491.5 mW/cm²) alone or in combination with one of three irradiance levels of 365 nm UV LED (180.1 mW/cm²,360.2 mW/cm², and 720.3 mW/cm²) for 60, 180 and 300 seconds. Log reduction plateaued at 4.95 Log_{10}.

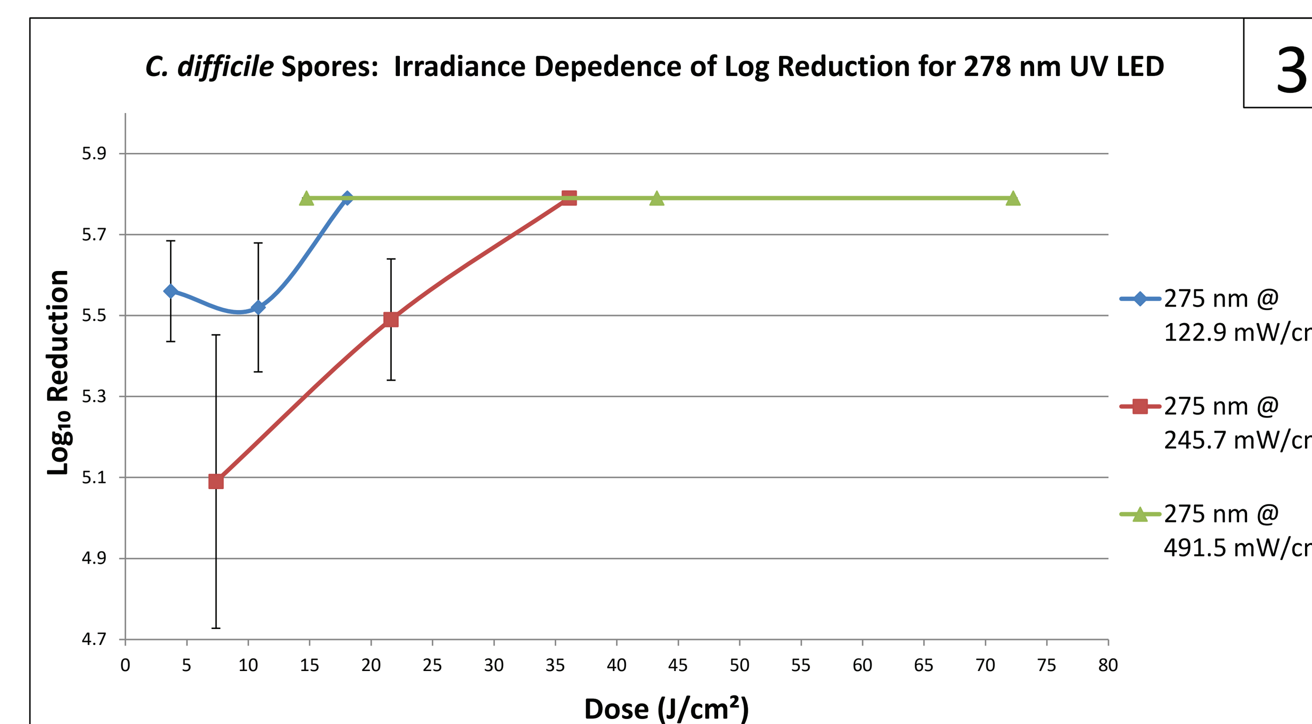

Figure 3: Log reduction of *C. difficile* CFU recovered from surfaces after hi-intensity 278 nm UV exposure. Spores were exposed one of three irradiance levels of UV LED (122.9 mW/cm², 245.7 mW/cm², and 491.5 mW/cm²) for 30, 88 and 147 seconds. Log reduction plateaued at 5.79 Log_{10}. (Previously presented during an oral talk.[1])

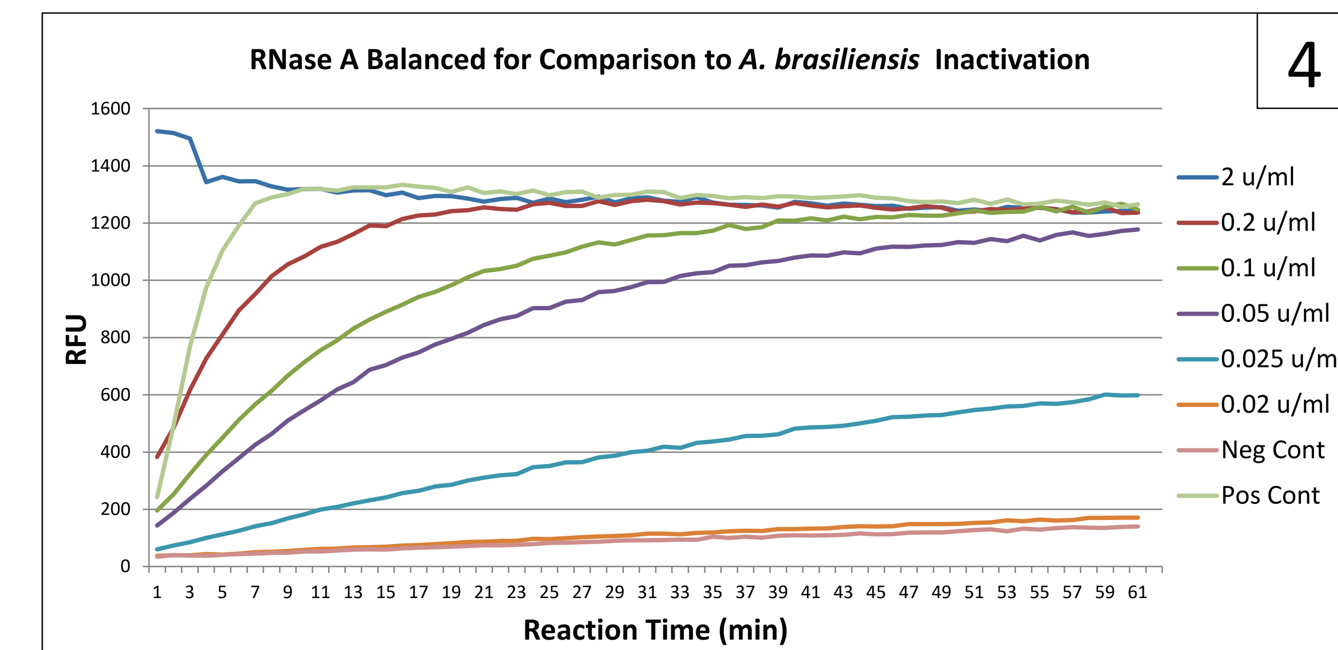

Figure 4: Inactivation of RNase A under conditions matched to *A.brasiliensis* 278 nm (491.5 mW/cm²) + 365 nm (360.2 mW/cm²) UV LED exposure that resulted in a 4.95 Log_{10} reduction in CFU, 60 second exposure duration. Six dilutions of RNase A were exposed to UV: 2, 0.2, 0.1, 0.05, 0.025, 0.02 U/µl. Enzyme activity was monitored post UV exposure for 1 hour with 1 minute time points (RNase Alert, IDT).

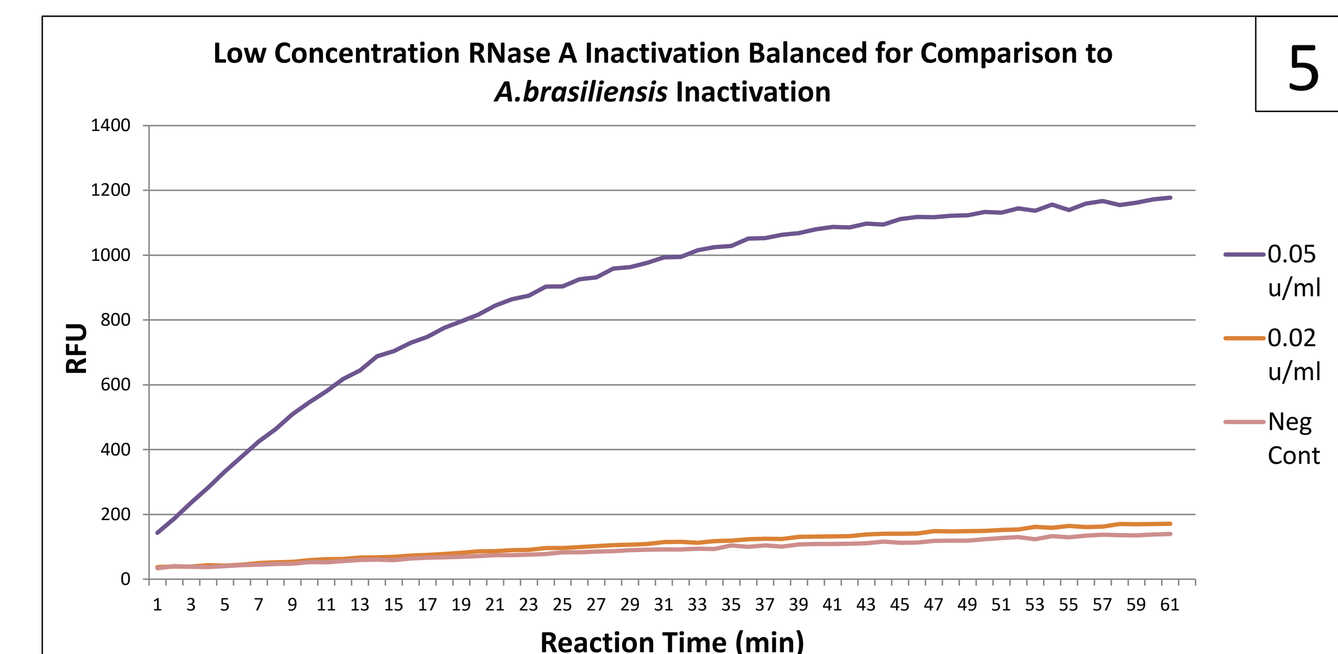

Figure 5: Low RNase A U/µl comparison to negative control for conditions matched to *A. brasiliensis* UV exposure conditions, as described in Figure 4. RFU is Relative Fluorescence Units. Each curve represents a single concentration of RNase A. Negative control is tracked by 0.02 U/µl RNase A, indicating enzyme inactivation. RNase A at 0.05 U/µl is significantly above the negative control. These concentrations were selected as predictive of the *A. brasiliensis* inactivation.

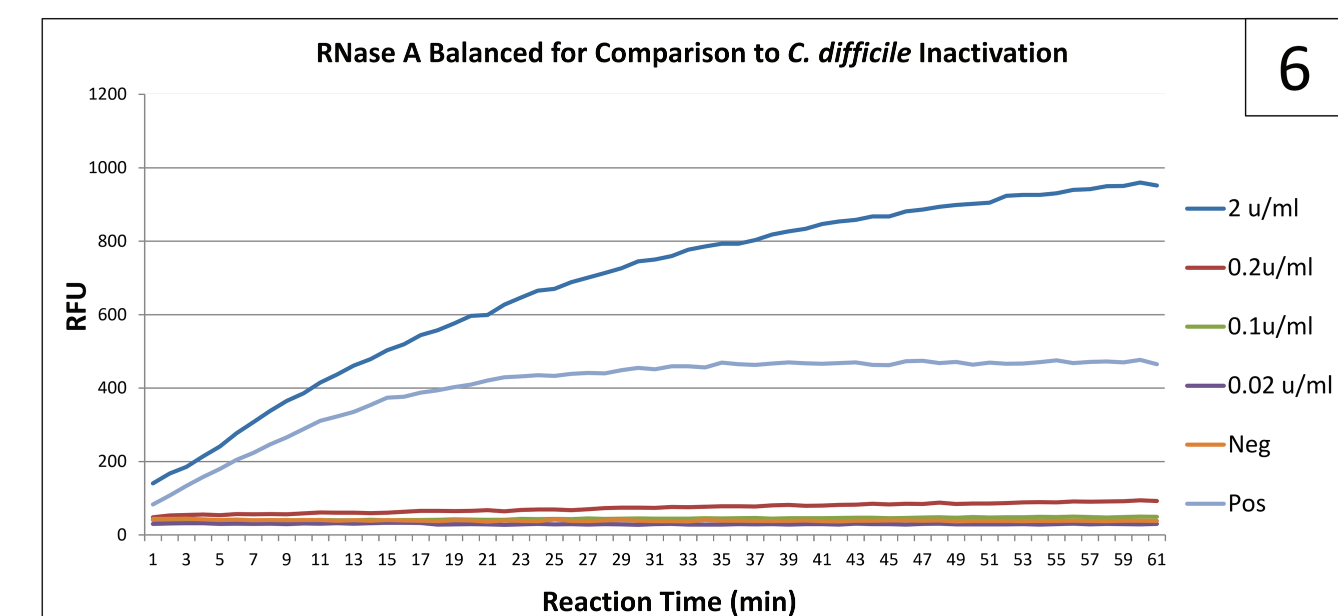

Figure 6: Inactivation of RNase A under conditions matched to *C. difficile* 278 nm (491.5 mW/cm²) + 365 nm (180.8 mW/cm²) UV LED exposure that resulted in a 5.79 Log_{10} reduction in CFU, 30 second exposure duration. Four dilutions of RNase A were exposed to UV: 2, 0.2, 0.1, 0.02 U/µl. Enzyme activity was monitored post UV exposure for 1 hour with 1 minute time points (RNase Alert, IDT).

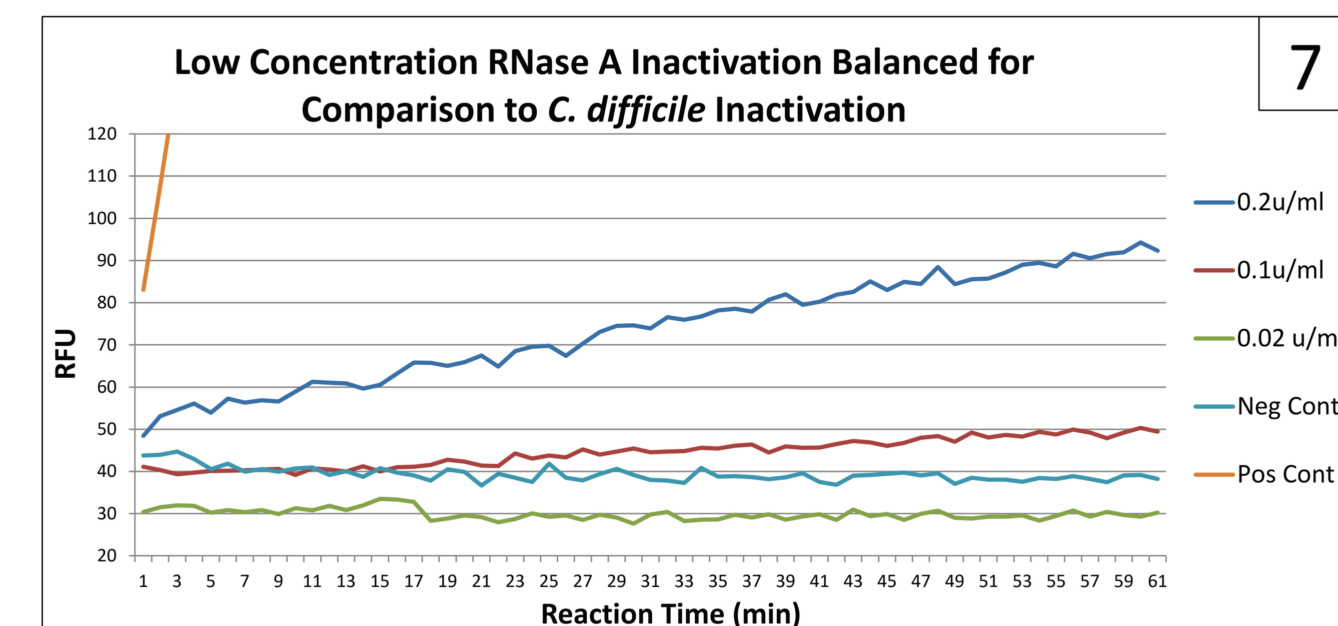

Figure 7: Low RNase A U/µl comparison to negative control for conditions matched to *C. difficile* UV exposure conditions, as described in Figure 6. RFU is Relative Fluorescence Units. Each curve represents a single concentration of RNase A. Negative control is tracked by 0.02 U/µl RNase A, indicating enzyme inactivation. RNase A at 0.1 U/µl is significantly above the negative control. These concentrations were selected as predictive of the *C. difficile* inactivation.

Literature Cited

Theresa L. Thompson, Jay Pasquantonio, "High-intensity UV LED Inactivation of *Clostridium difficile* Spores," SPIE Proceedings Volume 10863: Photonic Diagnosis and Treatment of Infections and Inflammatory Diseases II, June 2019.

Thompson, T, Taggard, K., and Pasquantonio, J, "Enzyme Activity Modulation by LED and UV LED," Poster presented at the annual SLAS conference, San Diego, CA., 2017.

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