Supplementary Materials: Searching for Activity Markers that Approximate (VBNC) *Legionella pneumophila* Infectivity in Amoeba after UV Irradiation

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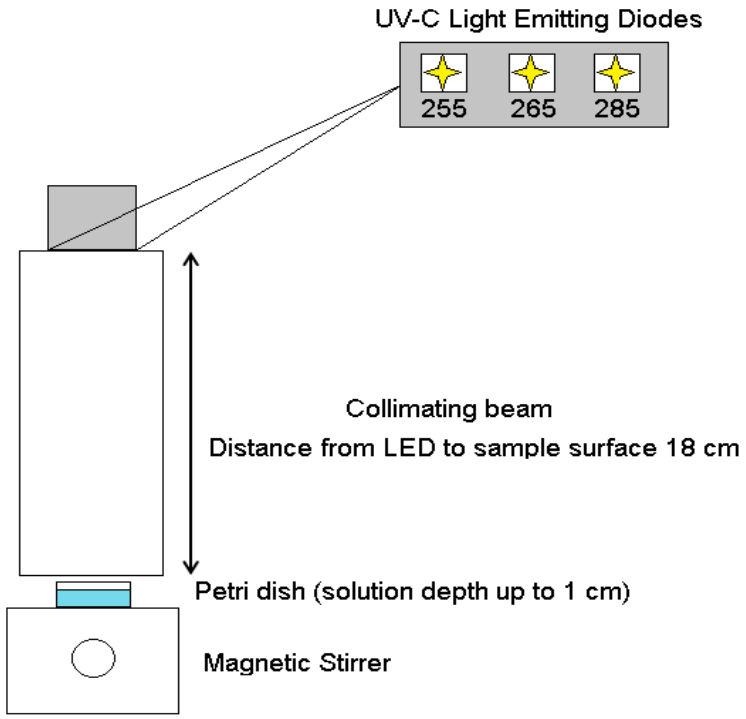
Supplemental Information

Schematic of collimating beam design



Equations used to calculate UV dose

$$E_{AVG} = 0.98\left[\frac{E_o}{L}\left(\frac{(T)^L - 1}{\ln(T)}\right)\right]$$

E_{AVG}: Average irradiance in water (mW·cm^{-2})

E_o: Incident irradiance (mW·cm^{-2})

L: water depth (up to 1 cm)

T: transmittance (1 – Absorbance at tested wavelength)

*Additional corrective factor required for measuring 265 and 285 nm wavelength irradiance were 0.54 and 0.96, respectively. Values multiplied to right side of equation to obtain average irradiance, correcting for radiometer measurement.

Exposure time: seconds

Dose: reported in mJ·cm^{-2}

Table S1. Photoreactivation control experiment data.

UV	Light	Photoreactivation	
		6 h	24 h
+	+	$1.7 \times 10^5 \pm 0.8 \times 10^5$	$2.5 \times 10^5 \pm 0.4 \times 10^5$
+	-	ND	ND

Table S2. Dose response statistics. Equations for best-fit lines generated in Figure 1 of main text, obtained from Student t-tests with null hypothesis difference of 0.

Wavelength (nm)	Equation of Best Fit	R^2	4-log Reduction	*P*-Value	6-log Reduction
256	$y = -0.0066x^2 - 0.3127x$	0.995	10.5	0.139	14.7
268.6	$y = -0.0245x^2 - 0.3398x$	0.9894	7.6	--	10.2
288.6	$y = -0.007x^2 - 0.1234x$	0.9971	16.7	<0.002	21.8

Table S3. Photoreactivation statistics. Analysis of photoreactivation data shown in Figure 2 of main text, obtained form running Student t-tests with null hypothesis difference of 0.

Wavelength	256 nm		268.8 nm		288.6 nm	
UV Dose ($mJ \cdot cm^{-2}$)	Log_{10} CFU	*P*-Value	Log_{10} CFU	*P*-Value	Log_{10} CFU	*P*-Value
10	5.3	--	5.0	0.27	5.8	0.13
20	4.7	--	3.4	0.03	5.3	0.20
30	3.5	--	2.0	0.01	4.9	0.01
40	1.8	--	0	0.02	4.5	0.001

Table S4. Statistical analysis. Data presented in Figure 3 of main text, obtained from running Student t-tests with null hypothesis difference of 0.

	P-Value			
Fluence ($mJ \cdot cm^{-2}$)	Esterase (N=5)	Live-Dead (N=4)	CTC (N=4)	ATP (N=4)
10	0.01	0.19	0.96	0.15
20	0.01	0.12	0.76	0.01
30	0.03	0.14	0.93	0.01
40	0.03	0.11	0.91	0.01

LED UV emission spectra

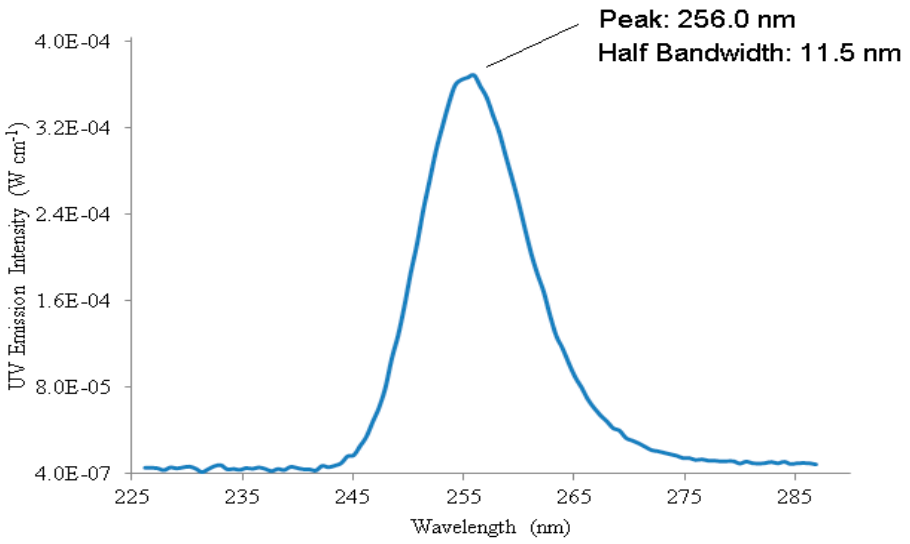


Figure S1. LED UV emission spectrum for 256 nm.

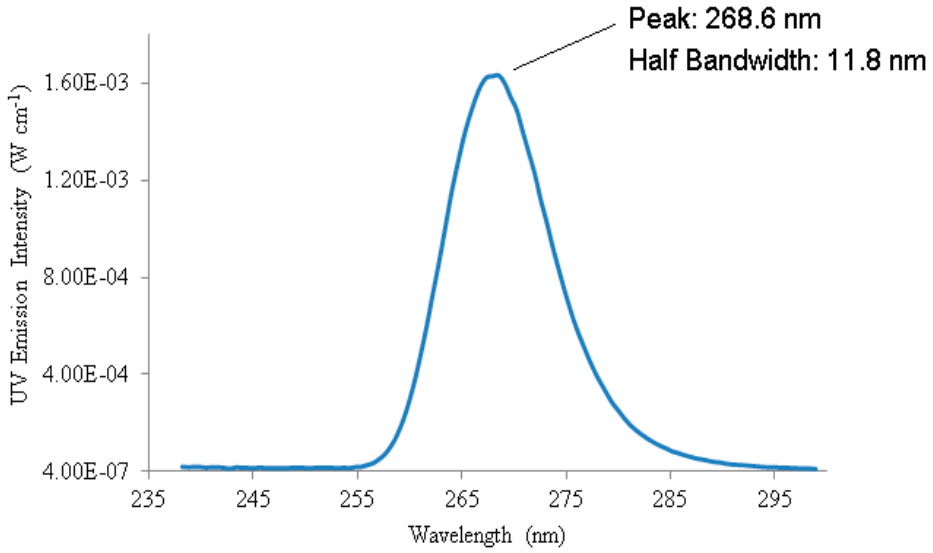


Figure S2. LED UV emission spectrum for 268.6 nm.

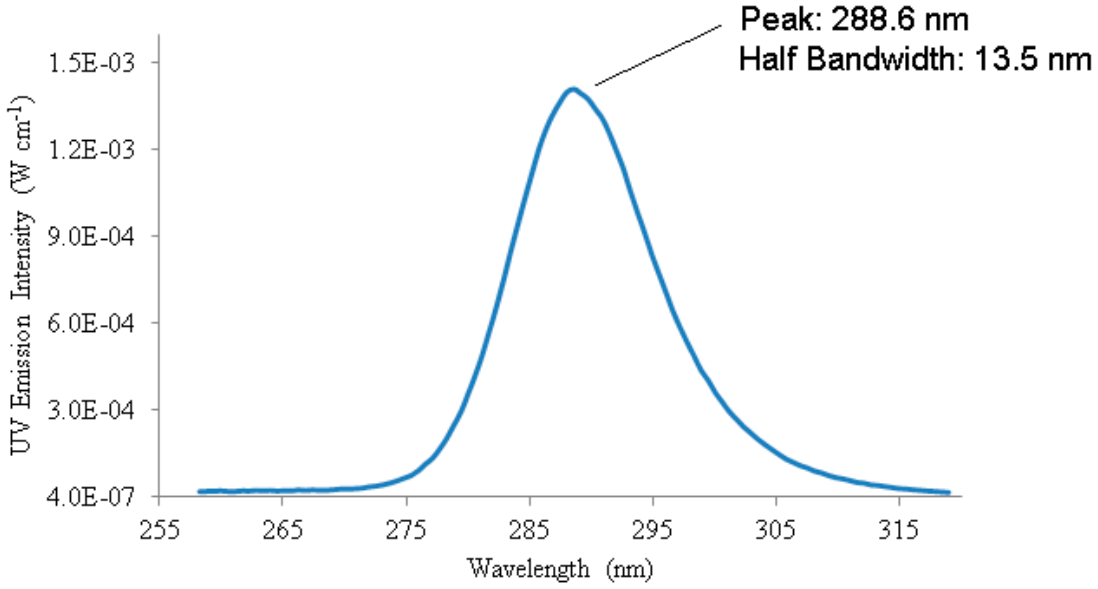


Figure S3. LED UV emission spectrum for 288.6 nm.

Confirmation of Intracellular *L. pneumophila* Following Infection

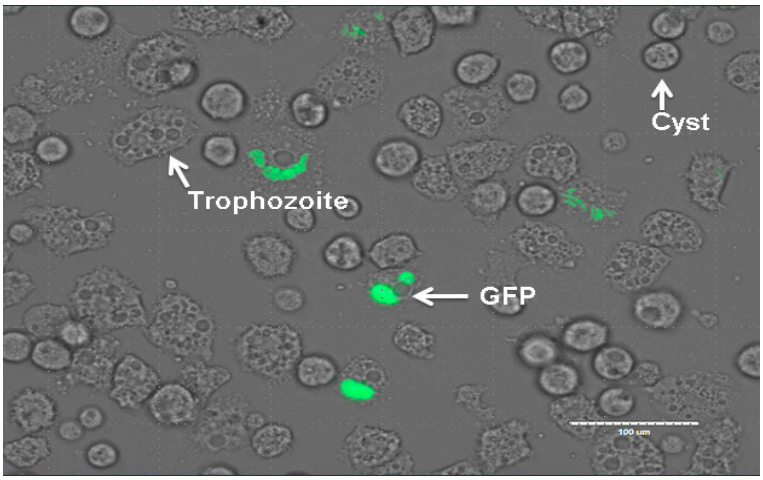


Figure S4. *Legionella* growth in amoeba. Fluorescent microscopic image of GFP expressing *L. pneumophila* (ATCC 33152) co-culture with *A. polyphaga* (ATCC 30461) following bacterial exposure to 16 mJ·cm^{-2} UV-C at 256 nm at 48 h after co-culture mix, incubated at 37 °C.

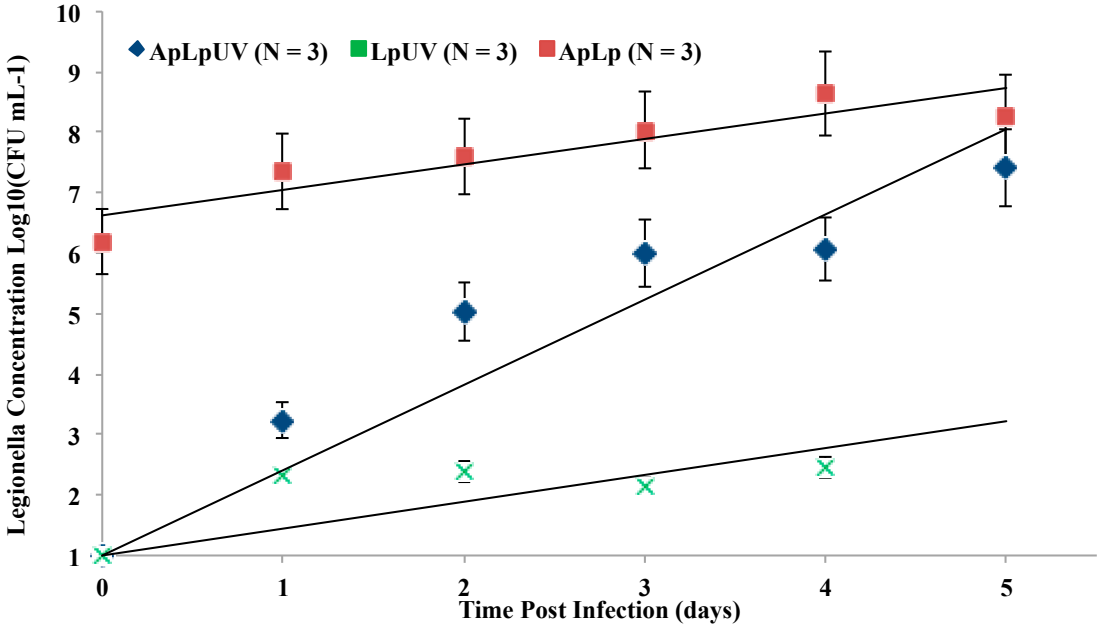


Figure S5. Amoeba co-culture controls. Control experiments for amoeba co-culture with 16 mJ·cm^{-2} exposed *L. pneumophila* and non-exposed cells, ApLpUV = reported in Table 1 of main article, LpUV = UV exposed *L. pneumophila* (no *A. polyphaga*) = 10^2 growth, may arise from nutrients in amoeba-preferred media source, ApLp = Non-UV exposed *L. pneumophila* (with *A. polyphaga*) = steady growth.

Controls confirm that *L. pneumophila* growth in *A. polyphaga* results in significant increase in bacterial concentration over 5 d experiment, with absence of FLA resulting in minor reactivation (control a) and no replication over a 4-d period for healthy *L. pneumophila*. Thus, the PYG 712 medium does not support *L. pneumophila* growth, *A. polyphaga* is required for propagation.

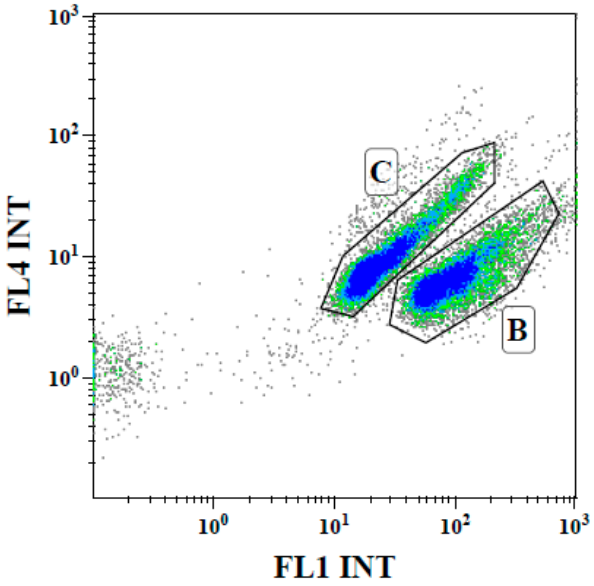


Figure S6. Live-dead flow cytometry plot. FCM scatterplot of live-dead staining with (C) dead population and (B) viable cell count based on relative FL-4 and FL-1 intensities.

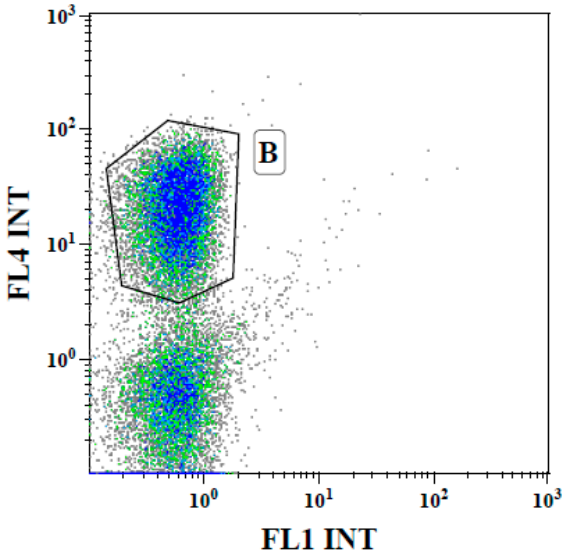


Figure S7. CTC flow cytometry plot. FCM scatterplot of CTC assay with (B) metabolically active cells that have reduced CTC to CTC-formazan.

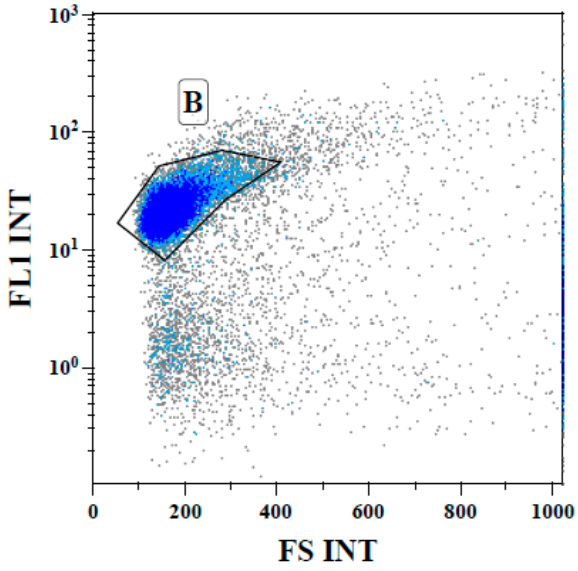


Figure S8. Esterase flow cytometry plot. FCM scatterplot of esterase assay with (B) metabolically active cells that have cleaved CFDA to FITC.

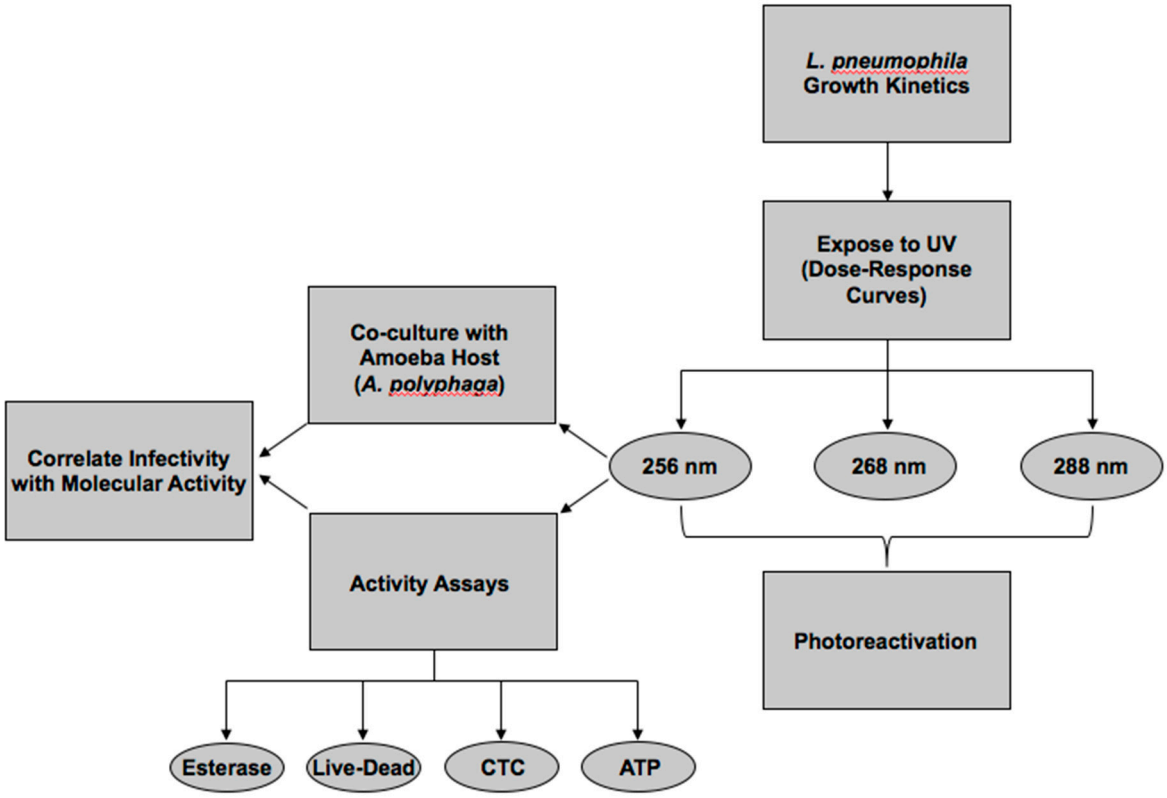


Figure S9. Methodology flow chart.