United States Environmental Protection Agency

Differences in the Inactivation of *Legionella pneumophila* Serogroups Using UV-C LED Technology in Drinking Water

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Office of Research and Development

Center for Environmental Solutions and Emergency Response

Homeland Security Materials Management Division

Acknowledgements

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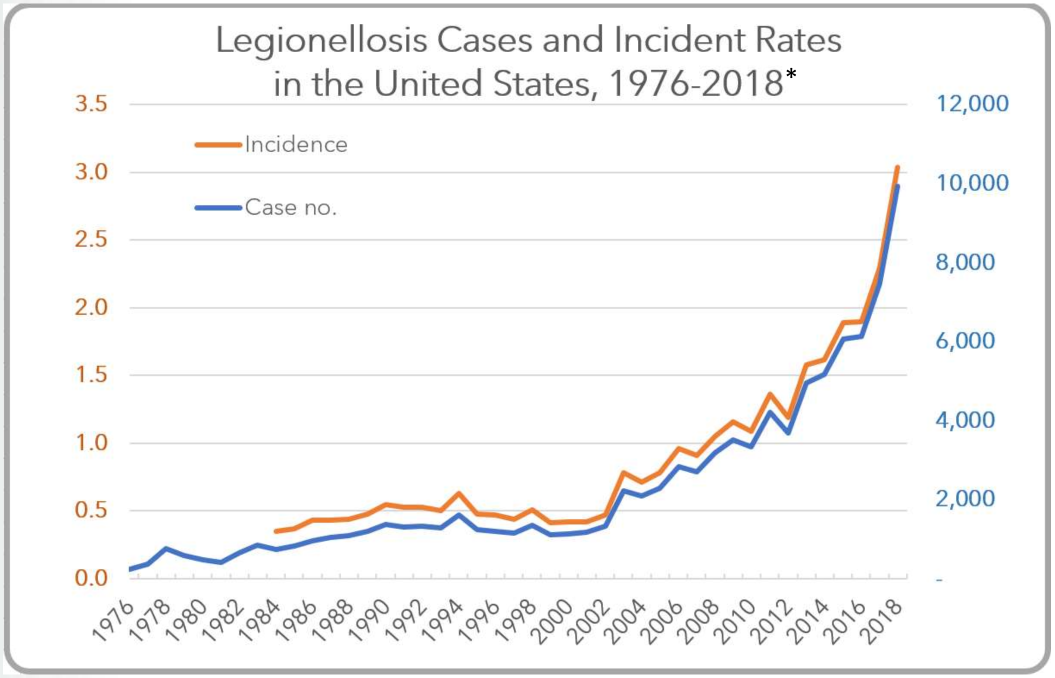
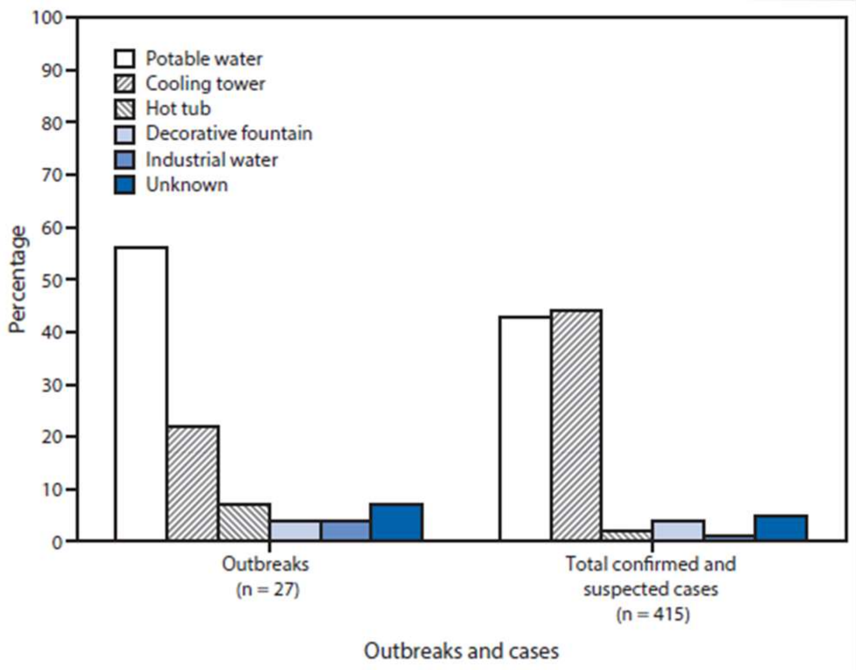
technologies

Legionellosis –
A Public Health and Economic Burden



*National Notifiable Diseases Surveillance System



- Incidence rate up from 0.4 to 3.0 cases per 100,000 people; exposure to potable water responsible for the majority of outbreak cases
- In 2014, LD cases had an estimated healthcare cost of $402 million (emergency department visits and hospitalizations)

Garrison, L. E., et al. (2016). MMWR Morb Mortal Wkly Rep.
Collier, S. A., et al. (2021). Emerg Infect Dis.

Legionella spp.

60+ species (serogroups)

L. adelaidensis	*L. feeleii* (2)	*L. monrovica*	*L. septentrionalis*
L. anisa	*L. geestiana*	*L. moravica*	*L. shakespearei*
L. beliardensis	*L. gormanii*	*L. nagasakiensis*	*L. spiritensis*
L. birminghamensis	*L. gratiana*	*L. nautarum*	*L. steelei*
L. bozemanae (2)	*L. gresilensis*	*L. norrlandica*	*L. steigerwaltii*
L. brunensis	*L. hackeliae* (2)	*L. oakridgensis*	*L. saoudiensis*
L. busanensis	*L. impletisoli*	*L. parisiensis*	*L. taurinensis*
L. cardiaca	*L. israelensis*	*L. pittsburghensis*	*L. thermalis*
L. cherrii	*L. jamestowniensis*	*L. pneumophila* (15)	*L. tucsonensis*
L. cincinnatiensis	*L. jordanis*	*L. qingyii*	*L. tunisiensis*
L. drancourtii	*L. lansingensis*	*L. quateirensis*	*L. wadsworthii*
L. dresdenensis	*L. londiniensis*	*L. quinlivanii* (2)	*L. waltersii*
L. drozanskii	*L. longbeachae* (2)	*L. rowbothamii*	*L. worsleiensis*
L. dumoffii	*L. lytica*	*L. rubrilucens*	*L. yabuuchiae*
L. erythra (2)	*L. maceachernii*	*L. sainthelensi* (2)	
L. fairfieldensis	*L. massiliensis*	*L. santicrucis*	
L. fallonii	*L. micdadei*	*L. saoudiensis*	

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Dresden panel of mAbs (17), groups Lp into sg 1 to 15

Lück, C. et al. 2013. Typing Methods for *Legionella*, p. 119-148. *In* C. Buchrieser and H. Hilbi (ed.), *Legionella*.

sg1, >80% clinical isolates

About ⅓ of these species associated with human disease

Lipopolysaccharide and Serogroup Relationship

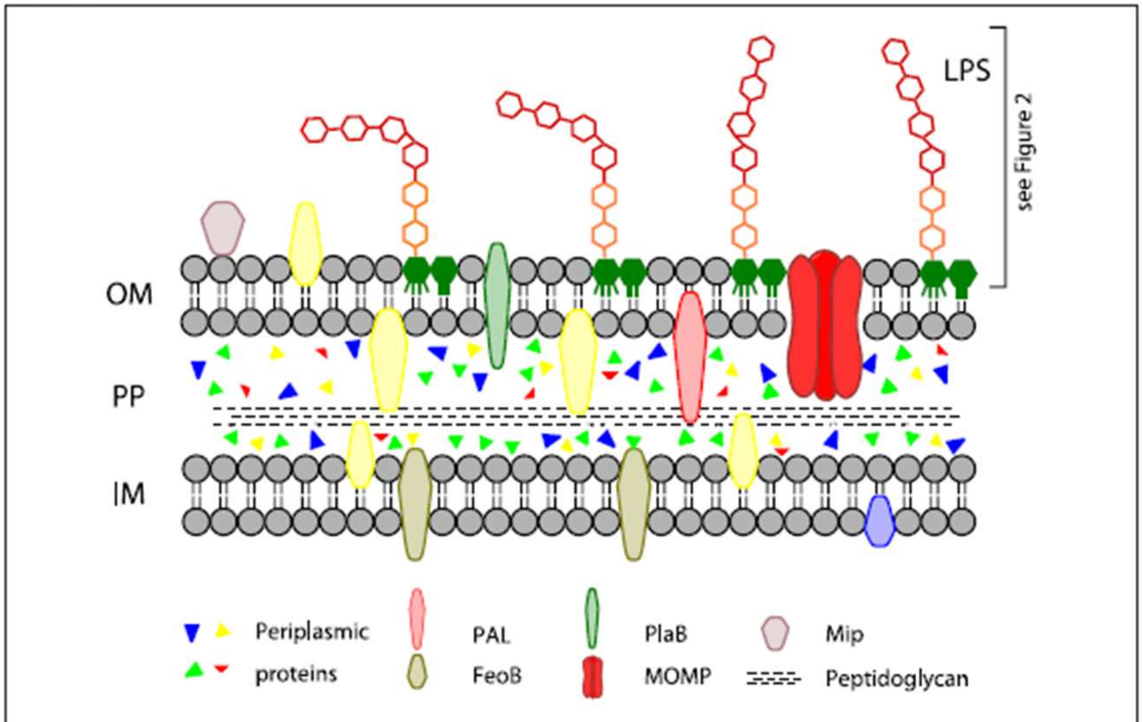


FIGURE 1 | Overview of the *L. pneumophila* cell envelope.

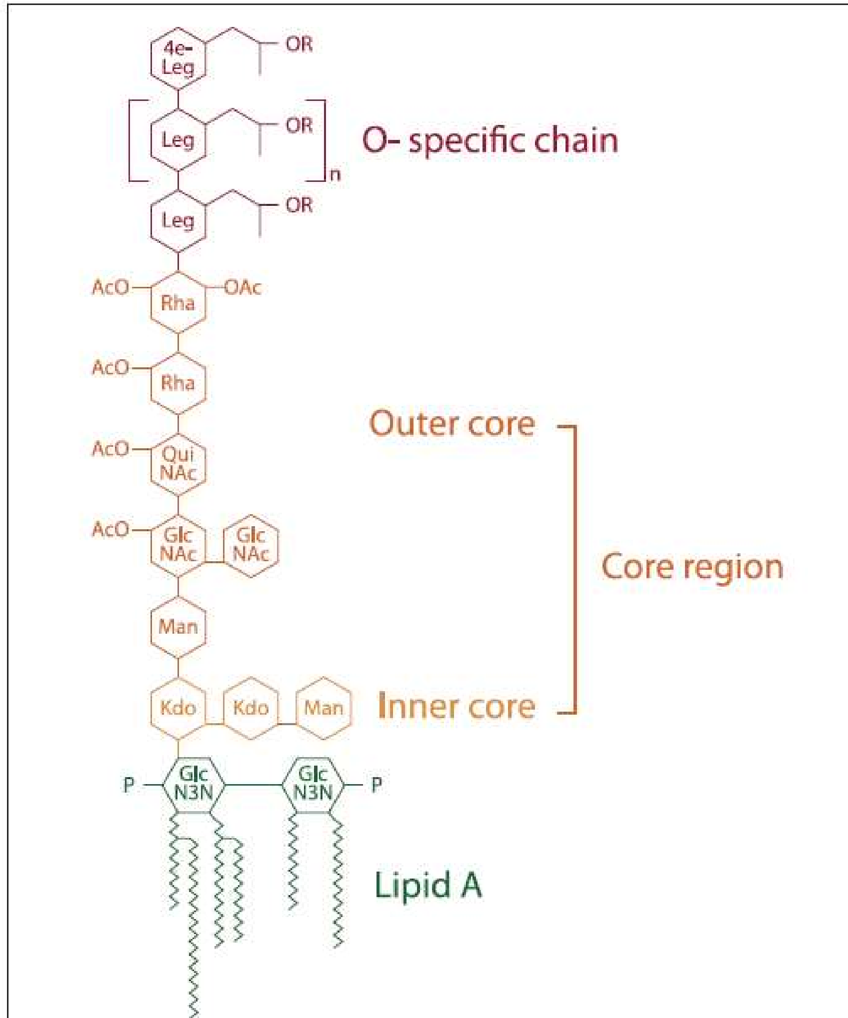


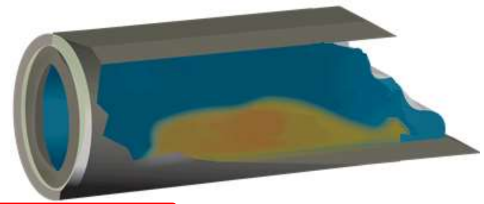
FIGURE 2 | Chemical structure of *L. pneumophila* LPS

- LPS: 3 structural domains
  - Lipid A
  - inner and outer core
  - O-antigen

Adapted from Shevchuk et al. 2011 Front Microbiol

Legionella's Niche Within Premise Plumbing

generation of respirable, DW-derived aerosols

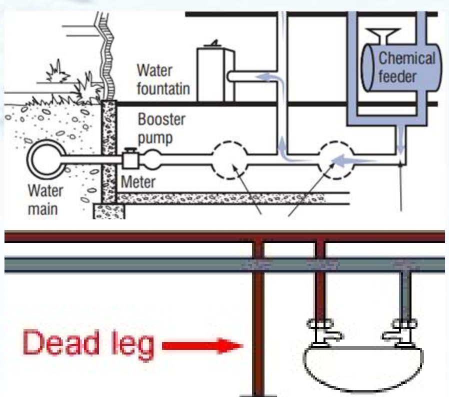


biofilms and sediments

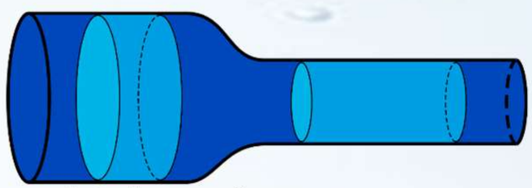
amenable growth temperatures and gradients

low to absent residual

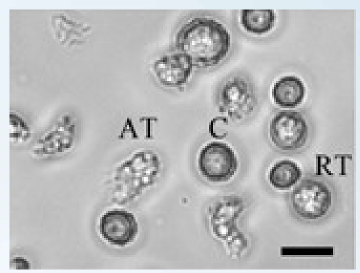
nutrients (metals, minerals, microbes, etc.)



cross-connections



high surface area to volume ratios



eukaryotic hosts

water stagnation

Summary of Previous *Legionella* UV Studies

- UV low pressure (LP) and medium pressure (MP) studies:
  - LP: *L. gormanii*; *L. longbeachae*; *L. pneumophila* sg1 (clinical, drinking water, other environmental isolates), sg7, sg8
  - MP: *L. pneumophila* sg1
  - 2-log reduction: 0.9-5 mJ/cm^2
  - 4-log reduction: 2.8-9.3 mJ/cm^2

- UV light emitting diodes (LED) studies:
  - *L. pneumophila* sg1 (clinical and drinking water isolates); *L. rubrilucens*
  - 2-log reduction: 1.1 mJ/cm^2 (UV-C); 25 J/cm^2 (405nm violet LED); 4 mJ/cm^2 (LP)

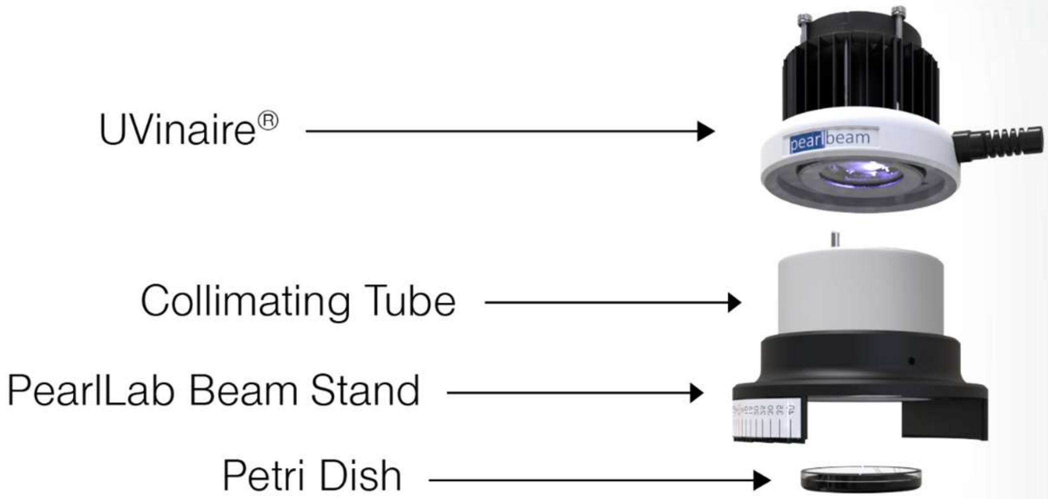
References for LP/MP studies:

Antopol & Ellner 1979 Appl Environ Microbiol
Cervero-Aragó et al. 2014 Water Res
Gilpin 1983 2nd Intl Leg Symp
Malayeri et al. 2016 IUVA News
Miyamoto et al. 2000 Microbios
Muraca et al. 1987 Appl Environ Microbiol
Oguma et al. 2004 Water Res
Wilson et al. 1992 AWWA WQTC

References for LED studies:

Carlson et al. *unpublished*
Hessling et al. 2018 Hosp Pract Res
Rattanakul & Oguma 2018 Water Res
Schmid et al. 2017 GMS Hyg Infect Control

Collimated Beam Method



fluence	255nm (UVT 79.7%)		265nm (UVT 86.5%)		280nm (UVT 91.7%)	
($mJ\ cm^{-2}$)	min	sec	min	sec	min	sec
0.5	0	17	0	4	0	2
1	0	34	0	9	0	3
2	1	7	0	18	0	6
5	2	48	0	44	0	15
10	5	36	1	29	0	31
16					0	49
34					1	44

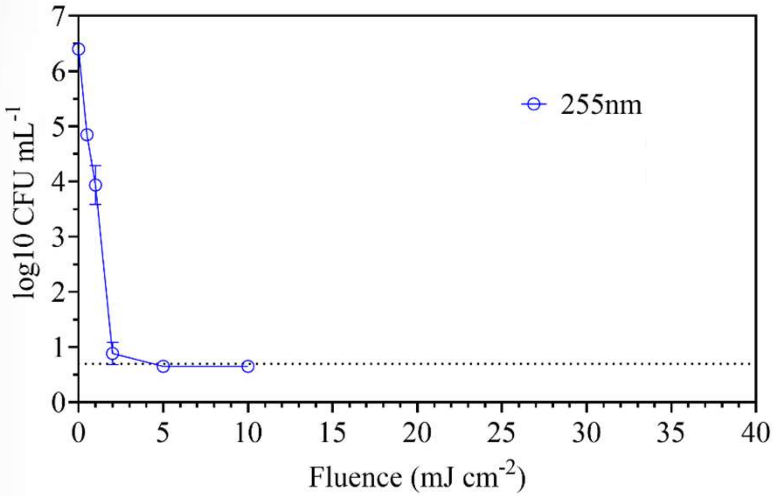
4 *L. pneumophila* strains:

- sg1 clinical
- sg1 drinking water
- sg4 clinical
- sg6 clinical

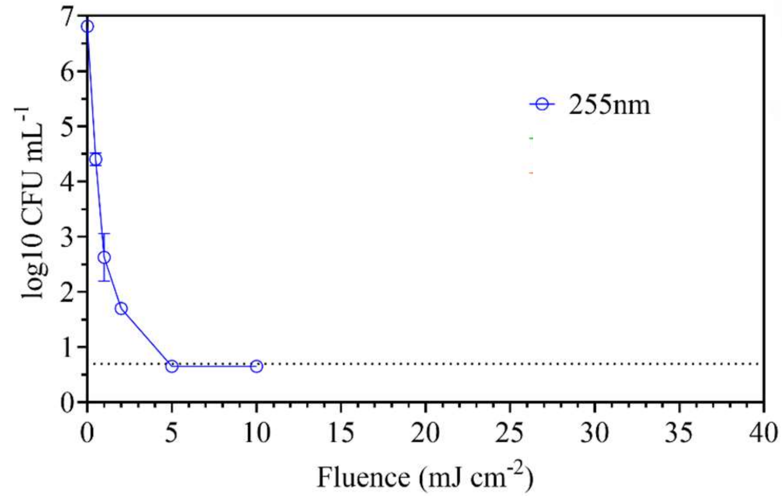
Three replicates

Collimated Beam Results – 255nm

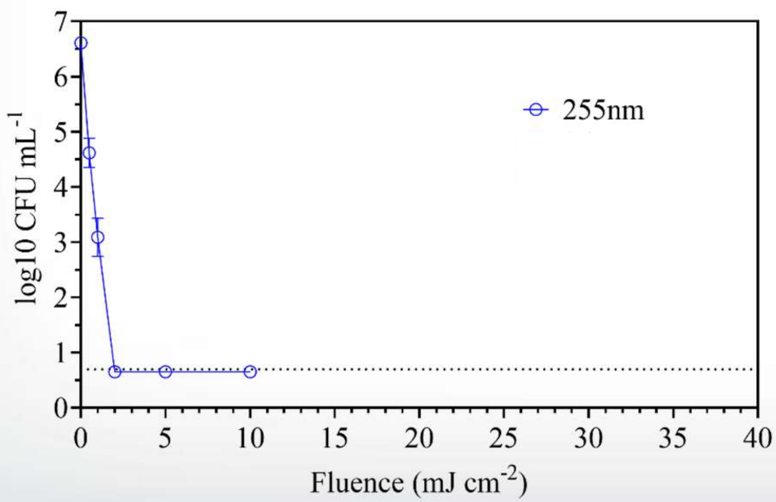
sg1



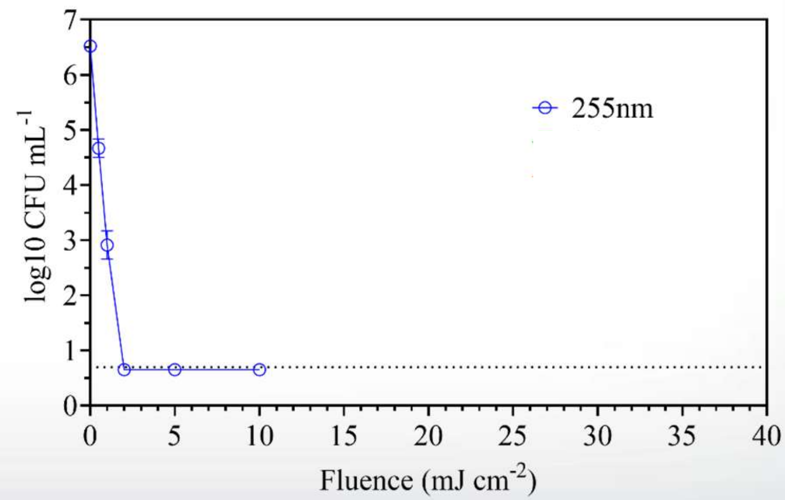
sg1 DW



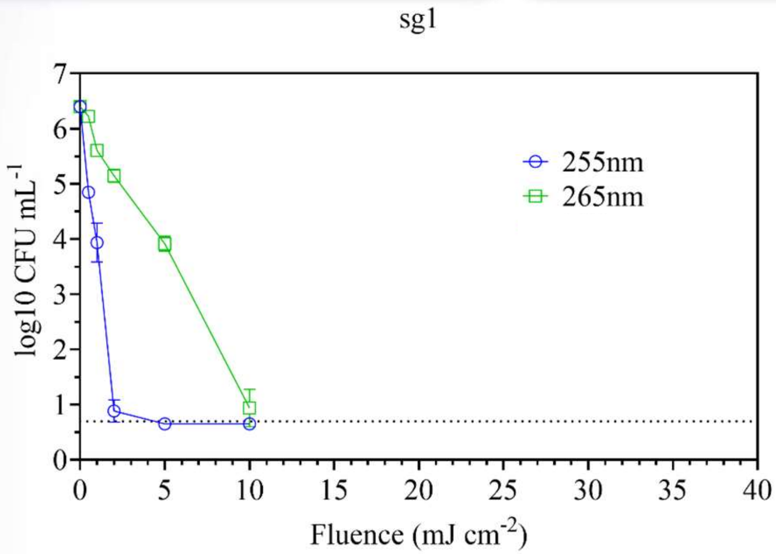
sg4

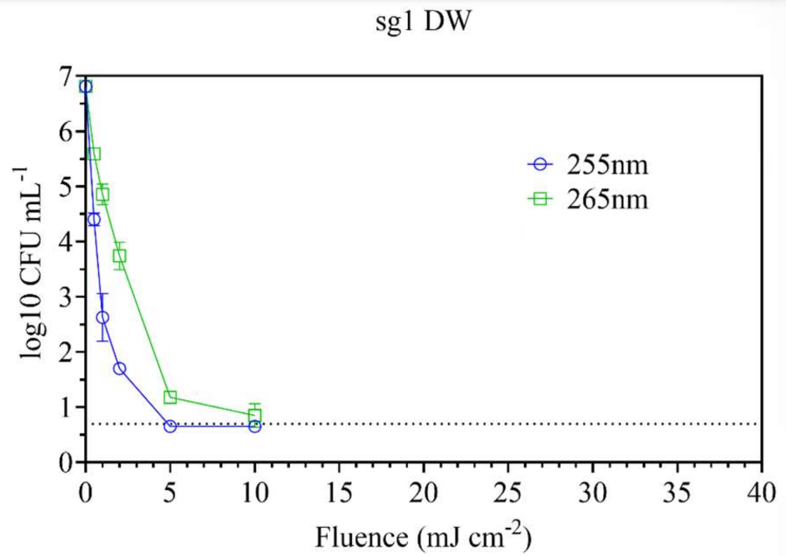


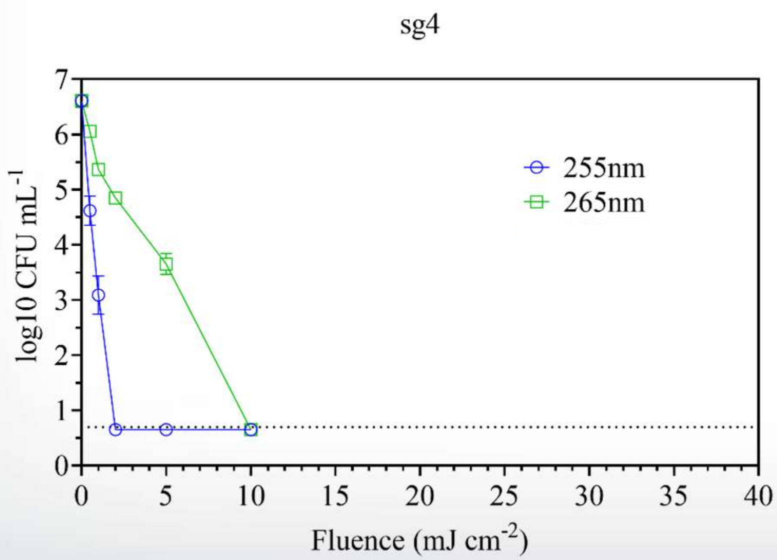
sg6

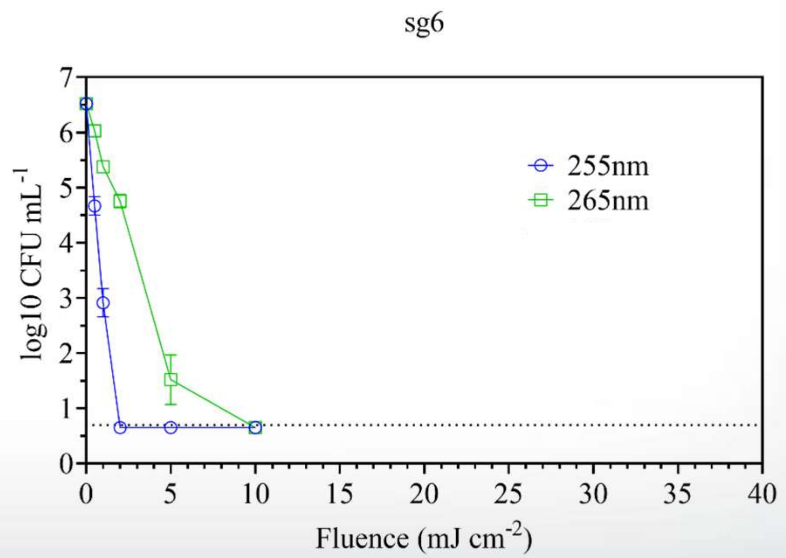


Collimated Beam Results – 265nm

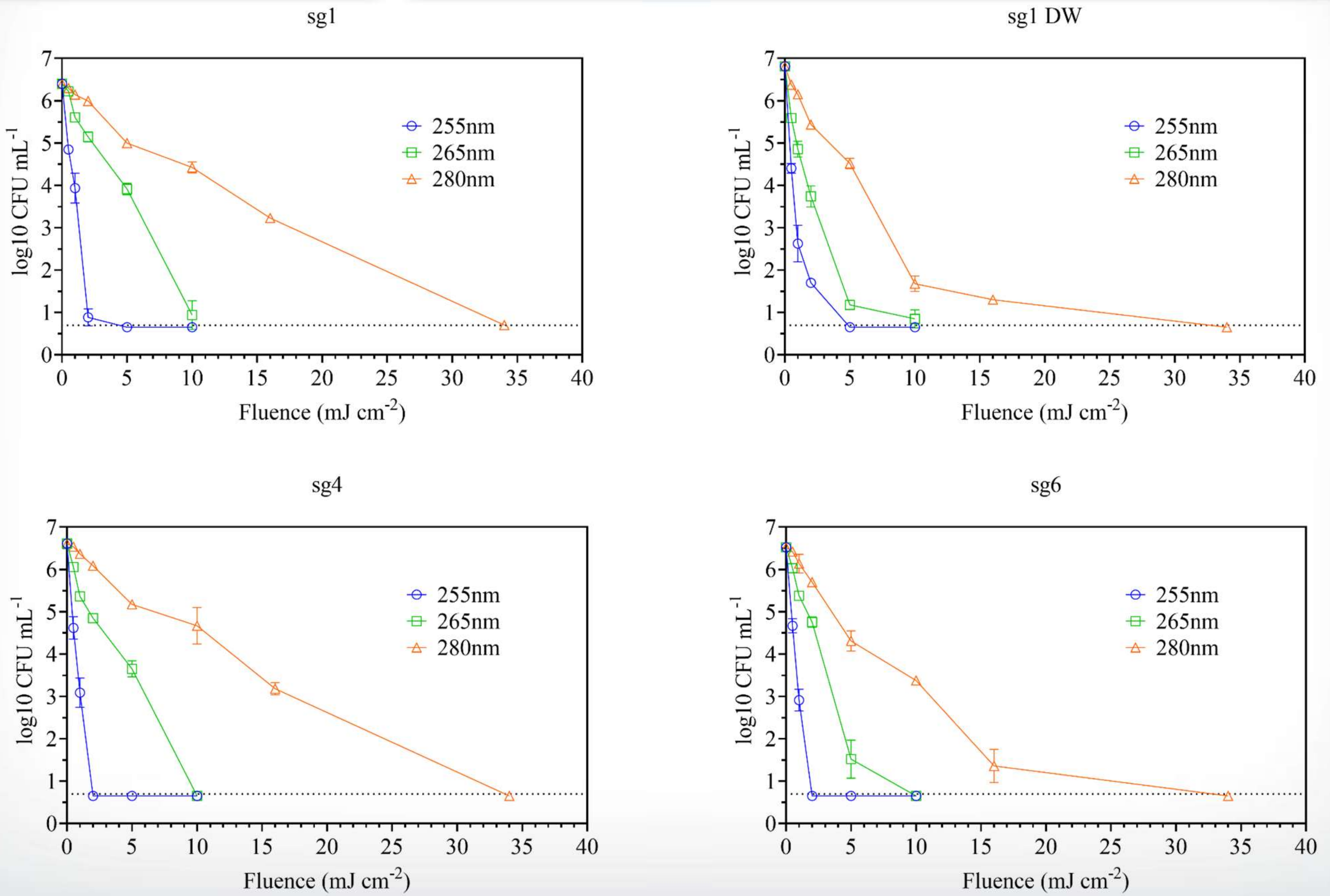




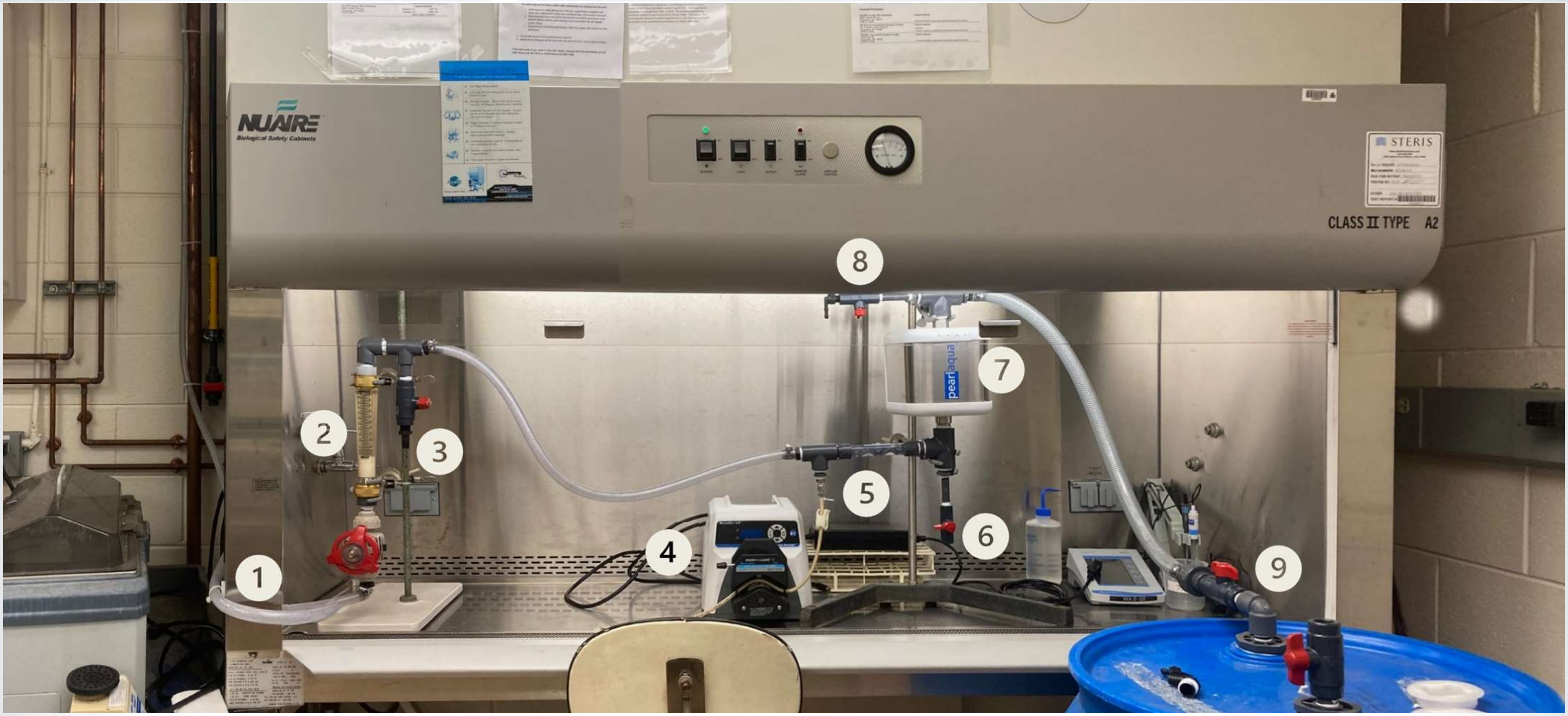




Collimated Beam Results – 280nm



Experimental Setup of the UV-C LED POE Device



1. Tap water source
2. Flow meter
3. Sample port
4. Pump/injection port
5. Pipe section with static mixer
6. Pre-treatment sample port
7. Water disinfection system
8. Post-treatment sample port
9. Liquid waste collector

Experimental Method

4 *L. pneumophila* strains:

- sg1 clinical
- sg1 drinking water
- sg4 clinical
- sg6 clinical

5 runs

- in sequence, turned on tap, Deca unit, pump, release *Legionella* inoculum tube clamp
- wait ~5s, collect 1L at the pre-treatment port (~30s)
- collect 1L at the post-treatment port (~30s)
- engage *Legionella* tube clamp and turn off pump
- flush influent then effluent port (~200mL ea)
- turn off Deca unit, then tap water

POE Device - Results

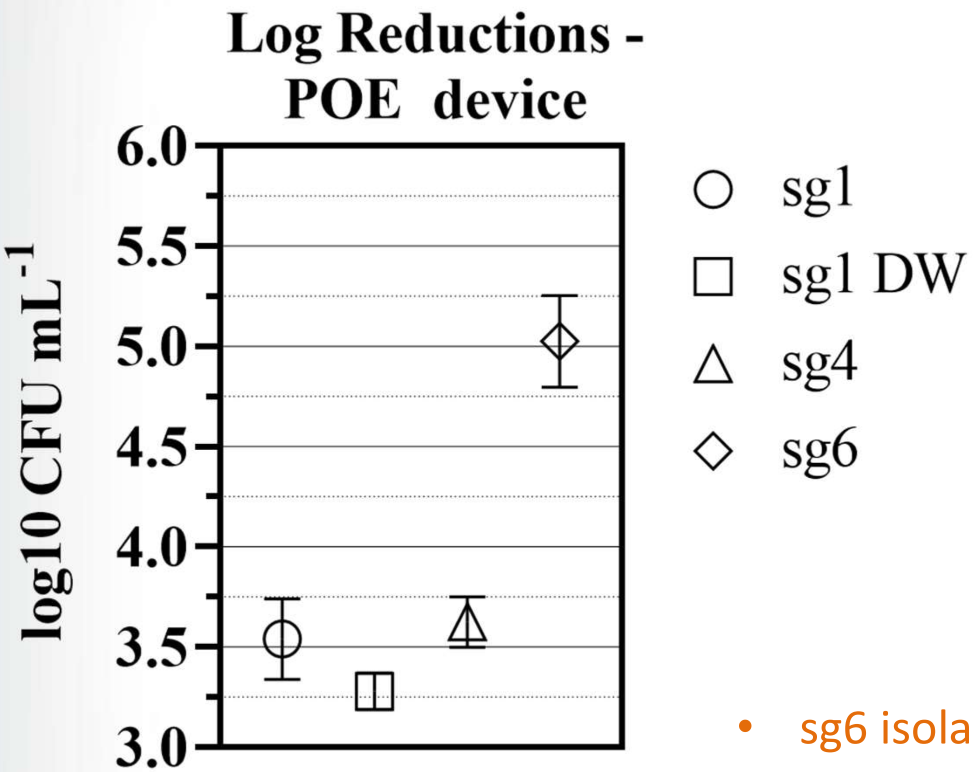
Table 1-1: Water quality specifications for optimal disinfection performance

Parameter	Description	Limits	Unit
UV-Transmittance* (UV-T)	Measure of how well UV light can travel through a fluid. Defined as the ratio of UV light intensity after passing through a liquid sample to the UV light intensity at the light source.	≥90	%
Particulate Size**	Dirt, dust, rust, sediment, and other solid particles	≤10	micron (μm)
Hardness***	Lime scale	7/120	gpg/ppm (mg/L)
Iron***	Rust stains	0.3	ppm (mg/L)

Table. Influent water quality parameters for each experimental run

Parameter (units)	*Legionella pneumophila* strain used			
	sg1	sg1 DW	sg4	sg6
pH	8.60 ± 0.05	8.70 ± 0.04	9.0 ± 0.09	9.1 ± 0.01
temperature (°C)	9.9 ± 0.1	15.1 ± 0.1	19.9 ± 0.1	17.1 ± 0.1
Hardness (mg/L $CaCO_3$)	130 ± 14	130 ± 14	120 ± 0	140 ± 0
Turbidity	0.25 ± 0.10	0.33 ± 0.00	0.64 ± 0.02	0.22 ± 0.00
% UVT 280nm	112.0 ± 0.1	93.4 ± 0.1	93.9 ± 1.3	111.3 ± 0.8
Free Chlorine (mg/L)	0.93 ± 0.00	0.92 ± 0.00	0.73 ± 0.00	0.92 ± 0.01
Total Chlorine (mg/L)	1.04 ± 0.00	1.04 ± 0.01	0.85 ± 0.01	1.05 ± 0.03
Ferrous Iron	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total Iron	0.05 ± 0.00	0.04 ± 0.01	0.00 ± 0.00	0.01 ± 0.00

POE Device - Results



Influent	Effluent	Log Reduction
5.9 ± 0.05	2.4 ± 0.47	3.5 ±0.46
6.3 ± 0.05	3.0 ± 0.17	3.3 ±0.21
5.5 ± 0.84	1.9 ± 0.81	3.6 ±0.13
6.1 ± 0.19	1.5 ± 0.95	4.6 ±0.91

Data presented as $\log_{10}$ ± SD CFU mL^{-1}

- sg6 isolate significantly more susceptible to inactivation
- Predicted UV dose at 2-2.5 gpm: ~80-100 mJ/cm^2
- Based on collimated beam studies, ~3-4 log reductions occurred ~10-15 mJ/cm^2

Main Summary Points

- Serogroup variations to UV inactivation – implications for DW treatment where known environmental strains are linked to clinical cases
- Simulating high contaminant loads, the POE AquiSense Technologies Deca device demonstrated at least 3-log reduction of planktonic *Legionella*
- Further studies needed to:
  - determine synergistic effects of chlorine, monochloramine, etc. and UV inactivation (also considering pH, temperature, other WQ parameters)
  - evaluate inactivation of shed biofilm-associated pathogens (e.g. microbial clumps, other suspended particles)

THANK YOU

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L. pneumophila sg1
drinking water isolate
grown on BCYE agar plates

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