The Value of UV for *Legionella* Control in Cooling Towers

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ABSTRACT: Excessive growth of *Legionella* in cooling towers and water systems can cause significant negative health effects. *Legionella* and free-living amoebae may be present together and amoebae can act as a shield for *Legionella*, protecting it from traditional chemical disinfectants. Ultraviolet (UV) disinfection offers a non-chemical treatment approach that is effective for reducing biofilm potential in cooling tower water and provides reliable protection against the spread of *Legionella* and inactivation of amoebae. However, all UV technologies are not created equal; low-pressure (LP) and medium-pressure (MP) UV lamp technology have different effects on a microorganism's mortality rate at different UV dose rates. In this comparative study, a proprietary MP lamp technology (Hydro-Optic™ [HOD]) manufactured by Atlantium Technologies was evaluated and determined to achieve 100% mortality of *Entamoeba Histolytica* (*E. histolytica*) at a dose of 8.8 mJ/cm^2, while LP UV systems, even at a dose of 90 mJ/cm^2, did not achieve 100% mortality. *E. histolytica* served as a *Legionella* host model in the study.

INTRODUCTION

Various studies have shown that 40-60% of all cooling towers harbor *Legionella* bacteria. Cooling towers are the largest and most common source of Legionnaire's disease outbreaks because of their risk for widespread circulation.

Although 90% of Legionella infections in humans are caused by *L. pneumophila*, there are 45 named species of *Legionella* of which 19 species have been documented as human pathogens.

A multinational study of community-acquired Legionnaires disease identified 508 culture-confirmed cases [Yu VL, Plouffe JF, Pastoris MC, et al., 2002]. *L. pneumophila* was responsible for the greatest percentage of cases (91.5%), followed by *L. longbeachae* (3.9%) and *L. bozemanii* (2.4%). The remainder of cases were due *to L. micdadei, L. feeleii, L. dumoffii, L. wadsworthii, and L. anisa*.

In a natural environment, *Legionella* lives in three forms: as a free-swimming form, as a biofilm and as an amoebic parasite (lives within amoebae).

Studies demonstrated that *L. pneumophila* can use free-living amoebae as host cells for intracellular replication [Skinner et al., 1983; Newsome et al., 1985; Fields, 1993]. *Legionella* and free-living amoebae may be present simultaneously in aquatic environments, hot systems and cooling towers. Thus, free-living amoebae may play a role in the amplification and protection of *Legionella* and could act as a vector in the transmission of Legionnaires' disease [Declerck et al., 2007].

LEGIONELLA IN COOLING TOWERS

The most common source of Legionnaires' disease outbreaks are cooling towers, primarily because of the risk for widespread circulation [Garcia-Fulgueiras et al., 2003]. Because of their mode of operation, cooling towers can create ideal conditions for microbial growth and they also deliberately require the creation of sprays and aerosols, which can be dispersed over a wide area if not controlled properly.

Cooling towers operate at temperatures that can provide optimal conditions for the growth of microorganisms in water (20-45°C, 68-113°F), including *Legionella*.

Other operating conditions contributing to the growth of *Legionella* in cooling towers include:

- High microbial concentration, including algae, amoebae, slime and other bacteria.
- Presence of biofilm due to high surface area, scale, sediment, sludge, rust and other organic matter.
- Presence of degraded plumbing materials that may provide nutrients to enhance bacterial growth.

Cooling towers must be properly disinfected and maintained to reduce the risk of *Legionella*.

CONTROLLING *LEGIONELLA*

Historically, biocides such as chlorine, chlorine dioxide, hypobromite, and ozone have been used for *Legionella* control in cooling towers. However, studies and previous data have shown that amoebae may adapt to biocides and as a result protect the *Legionella* against chemical disinfection treatment (i.e., *L. pneumophila* within protozoa may not be killed by the biocides).

When disinfection by biocides is insufficiently applied, the survival of *Legionella* and amoebae can promote rapid growth, and therefore, can be a source of Legionnaires' disease outbreak [Bargellini et al., 2011; Thomas et al., 2004].

UV disinfection is a cost-effective and efficient method of reducing the biofilm potential in cooling tower water and providing reliable protection against the spread of *Legionella*. UV disinfection is effective against all water-borne microorganisms, including those resistant to chlorine. UV does not fall under strict environmental discharge limits or require the handling, safety, risk mitigation, and storage of hazardous chemicals.

UV light has a strong germicidal effect that kills microorganisms by penetrating their cell membranes and damaging their DNA so they are unable to reproduce and die out.

Compared with other Gram-negative bacteria, *Legionella* are highly susceptible to UV irradiation [Antopol & Ellner, 1979]. Numerous studies have been undertaken to showcase the efficacy of UV treatment for *Legionella* control; the majority were conducted using LP UV lamp technology.

UV DOSE FOR *LEGIONELLA* CONTROL

There is a large body of published data (Table 1) related to the UV dose-response of various organisms [Wilson et al., 1992, Gregory B Knudson 1985, Antopol et al., 1979, Cervero-Arago S. et al., 2014] including *L. pneumophila* for 1, 2-, 3-, and 4-log reduction at 9.4 mJ/cm^2.

In addition, a dose of 30 mJ/cm^2 achieved 99.999 percent (5-log) reduction in 20 minutes [Muraca et al., 1987].

Table 1: UV Dose (mJ/cm^2) to Inactivate *Legionella*

Species	1-Log	2-Log	3-Log	4-Log
Legionella bozemani	0.5	1.1	1.9	
Legionella dumoffii	2.4	5.3	6.2	
Legionella jordanis	3	7.2		
Legionella longbeachae	1.4		6.3	
Legionella micdadei	3.8	6.2		
Legionella oakridgensis	3.4	5	6.2	
Legionella pneumophila	2.3	3.2	4.6	
Legionella pneumophila	0.92	1.84	2.76	
Legionella pneumophila	3.1	5	6.9	9.4
Legionella pneumophila	1.7-1.8		5.4-6.1	
Legionella wadsworthii	1.9	3.4	5	

Sources: Gregory B Knudson, 1985, Cervero- Arago S. et al., 2014, Antopol et at., 1979, Wilson et al., 1992

There are also several studies demonstrating that certain microorganisms, such as adenovirus, are more susceptible to broad spectrum polychromatic UV light produced by MP lamps (200-415 nanometers [nm]), compared with monochromatic UV light (254 nm) produced by LP lamps.

Data from the previous literature also show that the required UV doses produced by LP UV lamp technology for inactivation of amoebae are significantly higher than *Legionella* inactivation doses [Maya et al., 2003; Cervero-Arago et al., 2014; Chang et al., 1985].

MEDIUM PRESSURE UV DOSE TO INACTIVATE AMOEBAE

In a comparative study, Atlantium Technologies evaluated the effects of UV light (LP and MP) on the viability of *E. histolytica. E. histolytica* was chosen as a model *Legionella* host organism, due to having a UV resistivity similarity to other amoebae. The goal of the study was to determine if *E. histolytica* would need a lower UV dose to achieve inactivation using the proprietary MP HOD UV technology compared with LP UV technology.

The study showed that MP HOD UV technology achieved 100% mortality of *E. histolytica* at a dose of 8.8 mJ/cm^2, while LP UV systems, even at a dose of 90 mJ/cm^2, did not achieve 100% mortality.

The comparative study evaluated the reduction equivalent UV dose (RED) required for inactivation of *E. histolytica* by using a standard biodosimetry procedure in monochromatic (LP) and polychromatic UV lamp technology set up (MP HOD UV).

The *E. histolytica* UV dose response was measured using a collimated beam apparatus (CBA) device with standard LP UV and another CBA with MP HOD UV.

The UV dose calculation for LP UV CBA was performed according to the USEPA recommendations [USEPA, 2006], while the UV dose delivered (UV fluence) by the MP CBA was calculated according to A. Lakretz et al., 2010 [Lakretz et al., 2010] with slight modifications.

EXPERIMENTAL DESIGN

E. histolytica is a protozoan parasite responsible for a disease called amoebiasis. *E. histolytica* is transmitted via ingestion of the cystic form of the protozoa. Inside humans *E. histolytica* lives and multiplies as a trophozoite.

Exponentially grown trophozoites ($7x10^5$) were washed two times with phosphate buffer saline (PBS). One-half of the population was kept in PBS without being exposed to UV and the second half was exposed to UV at various intensity settings.

The viability of the trophozoites exposed or not exposed to UV was first determined by exclusion of the vital stain Eosin (0.2%). The exposed and non-exposed trophozoites were then transferred to *TYI-S-33* medium and they were cultivated for 24 h at 37^{o}C (99^{o}F).

After this period of culture, the cells were counted and the number of trophozoites in the non-UV-exposed inoculum was used as 100%.

RESULTS

The experimental results were graphically processed as UV doses vs. viability percentage (Table 2; Figures 1-2).

Table 2: % Viability as a Function of UV Dose

	UV Dose (mJ/cm^2)	% Viability (0h)	% Viability (24h culture)
	Control	100	100
LP Lamp	60.0	91	13
	72.0	74	11
	90.0	22	2
MP HOD UV Lamp	2.9	77	35
	8.8	35	0
	13.2	30	0
	17.5	36	0
	26.3	0	0
	35.1	0	0

Note: Results are only relevant to the proprietary Atlantium MP HOD UV lamp; performance for other MP based solutions cannot be assumed.

Figure 1: Effect of UV Exposure on *E. histolytica* Viability
(Immediate counting (0h)- eosin staining)
Purple- LP, Blue- MP HOD

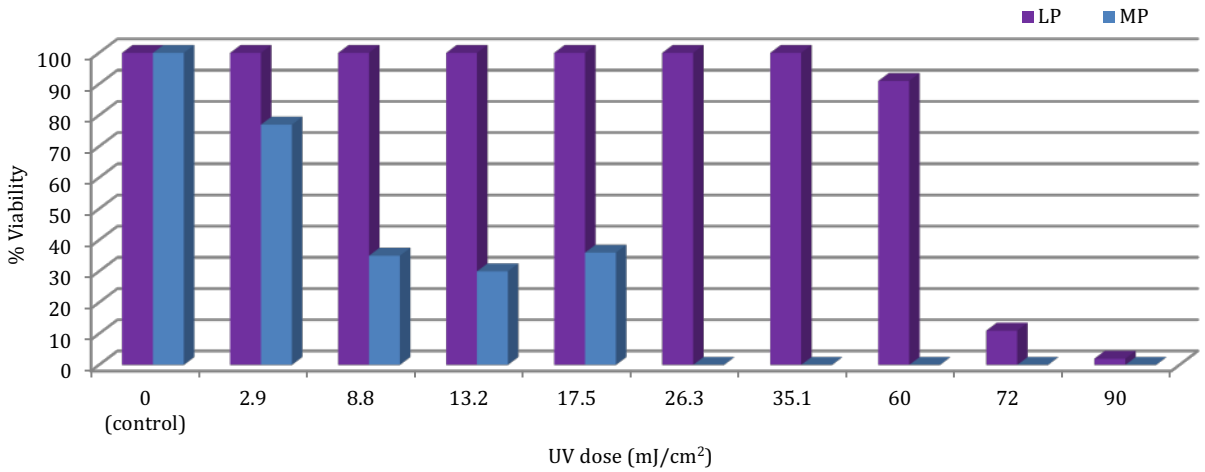

Figure 2: Effect of UV Exposure on *E. histolytica* Viability
(After 24h of Incubation)
Red - Control, Purple - LP, Blue - MP HOD

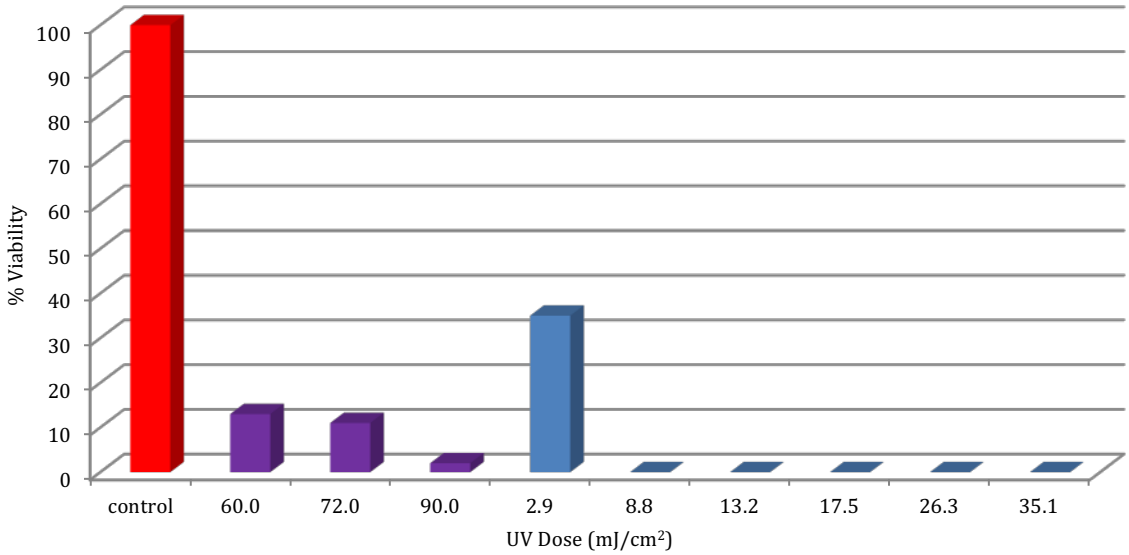

Figure 1: Immediate counting (0h) after UV treatment showed only 22% viability after illumination with the LP lamp at UV dose of 90 mJ/cm², while 100% mortality was observed after illumination with the MP HOD UV lamp at 26.3 mJ/cm².

Figure 2: After 24h of incubation, UV treated cells were still partially viable (2%) with LP lamp at 90 mJ/cm², and completely inactivated with the MP HOD UV lamp at 8.8 mJ/cm².

The MP HOD UV lamp is more effective in inactivation of *E. histolytica* compared with the LP lamp.

The enclosed LP results are in agreement with previous results of resistivity of amoebae to LP lamp [Maya et al., 2003; Cervero-Arago et al., 2014].

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CONCLUSIONS

There is a role for UV disinfection to control and treat for *Legionella*; however, all UV treatment systems are not equal. Amoebae can be resistive to UV doses produced from LP UV lamp system. The proprietary MP HOD UV system from Atlantium can treat amoebae and *Legionella* with low energy, achieving 100% mortality.