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MANAGING LEGIONELLA USING AN INNOVATIVE BACTERIAL CONTROL SYSTEM AND RAPID GENETIC LEGIONELLA TESTING

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Managing *Legionella* using an Innovative Bacterial Control System and On-site qPCR Testing

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Abstract

The greatest challenges for managing the risk of *Legionella pneumophila* in evaporative cooling water systems have been the lack of effective biocide treatment strategies and the lack of timely Legionella test results. The recent development of an on-site, on-line, rapid genetic test method for *Legionella* and an innovative plasma disinfection system (PDS) allows control of the population of all planktonic and sessile bacteria, including *Legionella*, other gram-negative bacteria and heterotrophic bacteria in a model evaporative cooling water system without the use of toxic non-oxidizing biocides. This paper summarizes the results of a laboratory study using a model cooling tower and on-site, rapid genetic test method for *Legionella* and results from the follow-on study of a cooling tower at a corporate headquarters of a medical device company that documented the validity of the results of the laboratory study. Researchers documented the value of the on-site, rapid test methods using a modified Legionella culture test.

Introduction

Plasma Disinfection System (PDS)

The patented Plasma Disinfection System (PDS) pairs two conventional biocidal technologies, copper-silver ionization and hypobromous acid, with a novel technology: non-thermal atmospheric plasma. Each of these three technologies have a different mechanism: non-thermal plasma damages planktonic Heterotrophic Aerobic Bacteria (HAB) and gram-negative bacteria; copper-silver ionization lyses the cellular membrane and poisons sessile bacteria and other single-celled organisms, including any Legionella bacteria incubating within the host organism; and the oxidizing biocide rapidly kills planktonic bacteria. This unique combination of technologies allows the PDS to be effective in both phases of the life cycle of Legionella bacteria: as a planktonic organism during the thriving phase; within a host organism during the reproductive phase.

Plasma. Plasma is the fourth state of matter that occurs when an electrical source with a sufficiently-high voltage frees electrons from atoms or molecules, creating an ionized gas in which ions and electrons coexist (micro-discharge filaments – "plasma streamers") at atmospheric pressure. By its nature, plasma is very unstable, resulting in the dissipation of the electrical energy as the cooling water flows between the two closely spaced electrodes. During this short period of electrical discharge, the high temperature of the plasma streamers effectively kills planktonic bacteria; the low rate of conductive heat transfer does not measurably raise the temperature of the cooling water. These plasma streamers also initiate chemical reactions at ambient temperatures that produce low concentrations of numerous short-lived ionized species, including several biocidal species: hydrogen peroxide (H_2O_2), ozone (O_3) and ultraviolet light (UV).

Copper-silver ionization (Cu-Ag). Copper-silver ionization is a very effective method to kill both planktonic and sessile bacteria. Yahya, et. al, documented the synergistic effect of the combination of copper and silver ions on bacteria viability.[1] Positively charged copper ions react

[1] Yahya MT, Gerba CP., "Water disinfection system and method." ChemAbstr 1992;118:87342.

with the negatively charged ions on the cell wall, damaging the integrity of the cell membrane and allowing the silver ions poison (bind to) the cellular protein (DNA and RNA) and the respiratory enzymes.
This disinfection technology is common in potable water distribution systems in facilities with immune-compromised occupants such as medical centers; however, copper-silver ionization systems are not very common in evaporative cooling water systems. From a physicochemical perspective,[2] there are several obstacles to apply this technology in evaporative cooling water systems. The solubility of copper ions decreases as the pH rises above 7.5, and copper ions react with free chlorine and organic compounds such as humic acid. Silver ions react with phosphate-based corrosion inhibitors, magnesium ions, and chlorides and the efficacy of disinfection by silver ions is sensitive to the temperature of the water. The most likely explanation for the lack of application of this technology in evaporative cooling water systems is that copper-silver ionization requires additional biocide technologies to control the bacteria population. This design has a separate copper and silver anode with fully-adjustable controls to generate and automatically dose copper and silver ions.

Oxidizing biocides. The electrolyzer produces sodium hypochlorite from a saturated brine solution. An automatic feedback control system uses an on-line oxidation-reduction potential meter (ORP) for feedback control of a mixture of sodium hypochlorite and hypobromous acid to control planktonic bacteria. The PDS system includes an on-line amperometric free chlorine sensor to monitor the free halogen residual.

The PDS is a fully integrated, skid-mounted assembly with a central programmable logic controller (PLC) that controls the onboard subsystems: plasma generation, copper ionization, and silver ionization. The PLC also controls the flow systems (valves and internal pump) and records operating parameters (plasma parameters (e. g. power, probe temperature) fan speed, pump speed, inlet and outlet water pressure, water flowrate, inlet and outlet water temperatures, leak sensor) and water quality parameters from on-line sensors (conductivity, pH, ORP, temperature).
Figure 1 show the prototype of the master cabinet that includes the plasma device and the copper and silver ionization units; **Figure 2** shows a close-up of the user interface. In January 2021, the research team conducted the laboratory study on this prototype unit. The field study site had two prototype units to treat an evaporative cooling water circuit that has a higher volume than the lab study. Each cabinet has an Uninterruptible Power Supply (UPS) to guard against intermittent voltage fluctuation or power loss.

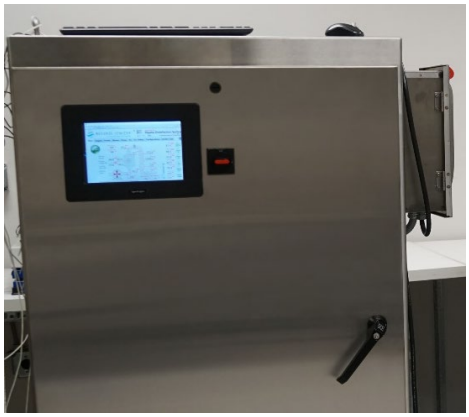

Figure 1 – PDS Plasma/Cu-Ag Ionization Unit

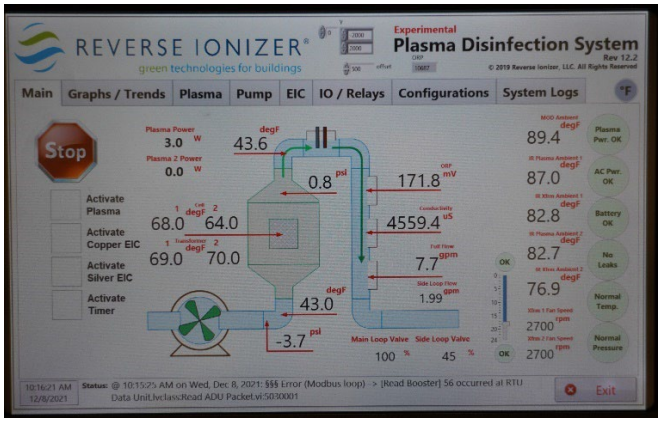

Figure 2 – PDS User Interface

[2] Thurman RB, Gerba ChP., "The molecular mechanisms of copper and silver ion disinfection of bacteria and viruses." Crit Rev Environ Control 1989;18(4):295–315.

Figure 3 shows a prototype stand-alone device that generates oxidizing biocide using an electrolytic process to convert a saturated brine solution to hypochlorous acid. This batch process recycles the product stream through the electrolysis chamber to minimize the concentration of free chlorides. A reservoir of liquid bromine is the source of hypobromous acid. In this prototype model, an onboard PLC manages the production of hypochlorous acid and schedule the feed of a mixture of hypochlorous and hypobromous acid. This system uses IoT technology to create a cloud-based data archive.
Researchers are currently building a pre-production model that will combine the plasma device, the copper silver ionization unit and the generation of oxidizing biocide into a single master cabinet with an onboard PLC.

Figure 3 – Electrolyzer - Oxidizing Biocide Generator

The process flow diagram in **Figure 4** details the sequence of treatment for the side stream sample of cooling water flowing through the PDS: non-thermal plasma, followed by Cu-Ag ionization, followed by oxidizing biocide. The typical operating mode is continuous treatment using non-thermal plasma and batch treatment of Cu-Ag ionization and oxidizing biocide.

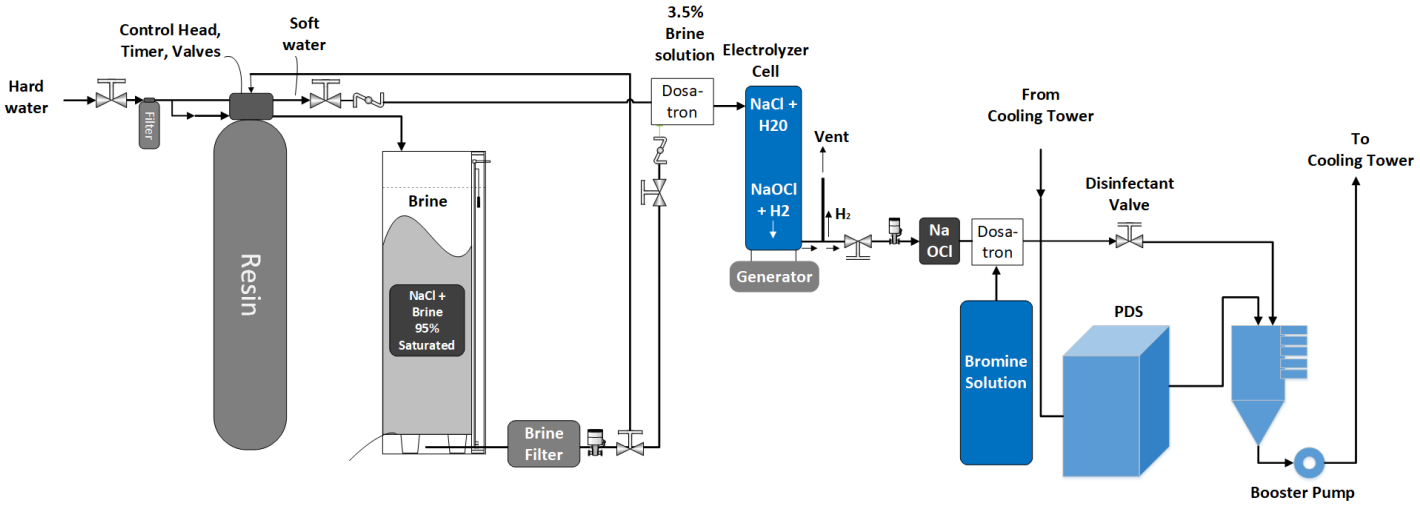

Figure 4 – Process Flow Diagram: PDS and Oxidizing Biocide Generator

Discussion

Background. Prior to the start of the field study, researchers conducted a laboratory study with full-scale PDS and Electrolyzer systems and a model cooling tower installed outside the laboratory. The study had two parts: inoculation and test. The objective of this study was to demonstrate the efficacy of several combinations of biocidal technologies.
The test conditions simulated the hydraulic, thermal, and environmental conditions of a comfort cooling tower, except for chemical additives for control of deposits and corrosion. Because water treatment chemicals serve as nutrients for bacteria, researchers compensated of water treatment

chemicals by adding a different nutrient, nitrate, and inoculating the system with *Legionella pneumophila* bacteria. Researchers operated the cooling water system for approximately one week to ensure a sustainable concentration of Legionella bacteria. The concentration of *Legionella pneumophila* at the beginning of the test phase was 1300 CFU/mL.

The duration of the test phase was 136 hours: plasma-only: 56 hours; plasma with Cu:Ag ionization: 24 hours; plasma with Cu:Ag ionization and oxidizing biocides: 56 hours. **Figure 5** shows that the information provided by the field paddle test method for gram-negative bacteria provides useful information about the efficacy of the non-thermal plasma and copper: silver (Cu:Ag) ionization technology to control sessile bacteria.

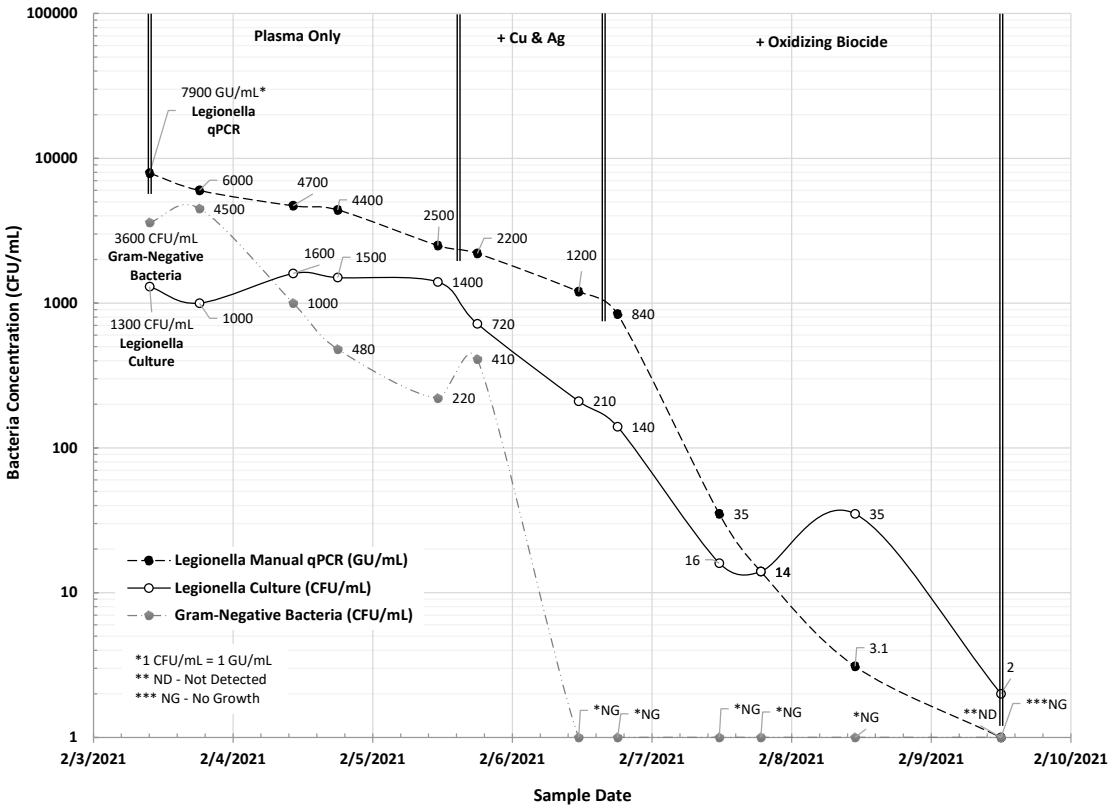

Figure 5 – PDS Performance in Laboratory Study (February 2021)

A complete review of the laboratory study was published in "the ANALYST."[3]

Field Study Site. The field study site is the headquarters of a medical device company located in northern New Jersey. This site has two four-story office buildings and one-story cafeteria; each building has a dedicated evaporative cooling water system. The cooling tower in the older office building 30,658 m^2 (330,000 ft^2) served as the test system. This Marley Cooling Tower has three (3) cells, each rated for 277 metric tons (295 US tons) (3350 lpm (885 gpm) recirculating flowrate) that serves three (3) centrifugal chillers, each rated for 281 metric tons (310 US tons).

PDS Test System. The two PDS systems used in this study are full-scale pre-production models. Key specifications include the electrical requirements [208V, single phase, 20A, 50/60 Hz], nominal energy requirements [4.16 KW], ambient conditions [conditioned space <40° C (105°

[3] Huchler, Loraine, A., Fraser, Desmond; "Can Onsite qPCR Testing Improve Management of Legionella Infections From Cooling Towers?" the ANALYST, Vol. 28, No. 3, Summer 2021, pp. 8-18.

F)], cooling water sample flowrate (75.7 lpm (20 gpm)), dimensions [81.5 cm x 61 cm x 152.5 cm (32" x 24" x 60")] and weight [340 kg (750 lbs)]. A second skid, integrated with the PDS programmable logic controller (PLC) via Modbus communications, includes a bromine storage tank and the electrolysis equipment [181 kg (400 lbs), 145 cm X 94 cm X 183 cm (45" X 37" X 72")].
Researchers installed the PDS and Electrolyzer units in the mechanical room near the chillers and the recirculating cooling water pumps. A 1.27 cm (½") diameter tap on the cooling water return served as the sample for the PDS; a 1.27 cm (½") diameter tap on the cooling water supply served as the injection of the PDS treatment for the cooling water system.

Water Treatment Program. Prior to the start of this field study, the chemical treatment program included intermittent feed of three chemicals: a deposit and corrosion control agent (phosphonate/polymer dispersant), an oxidizing biocide (stabilized bromine) and an algaecide (quaternary ammonium). This chemical water treatment program did not include a non-oxidizing biocide. The dosing protocol for all three chemicals was intermittent feed.
At the start of the inoculation phase of the field study (June 2021), the water treatment service representative terminated the feed of the oxidizing biocide and the algaecide; the incumbent water treatment supplier continued to feed the deposit and corrosion control chemical (phosphonate/dispersant product). The PDS provided all of the bacteria control.

Test Methods and Analytical Measurements. Table 1 lists the analytical measurements for this study. This list does not include the biocidal ionized species (hydrogen peroxide (H_2O_2), ozone (O_3) and ultraviolet light (UV)) formed from the reaction of plasma with cooling water because these biocides are short-lived and occur in very low concentrations.

Table 1 – Analytical Measurements

Measurement	Equipment	Test Method Range
Heterotrophic Aerobic Bacteria	Laboratory Spread Plate Culture	<1 – 10^9 CFU/mL[4]
Heterotrophic Aerobic Bacteria[5]	Field Paddle Tester	10^2 - 10^6 CFU/mL
Sessile Bacteria	Semi-quantitative Test	20 – 1.75 x 10^6 CFU/mL
L. pneumophila, all sero groups	Laboratory Modified Culture	<1 – 300 CFU/mL[6]
L. pneumophila, all sero groups	Field qPCR Test – manual method	1 – 80,000 UG/mL
Legionella, all sero groups	Field qPCR Test – on-line method	5 – 5,000 UG/mL
ATP, Free & Total	Field ATP Test	0 – 9,999 RLU
Free Halogen	Spectrophotometer	0.04 - 4.00 mg/L
Halogen, pH	On-line Amperometric Free Halogen Analyzer	0.04 - 4.00 mg/L, 0 - 14
Copper	Spectrophotometer	0.06 - 5.00 mg/L
Silver	Spectrophotometer	0.01 - 0.25 mg/L
Total Hardness	Spectrophotometer	3 - 100 mg/L as $CaCO_3$
Conductivity	On-line Conductivity Sensor	10 - 10,000 µS/cm
Temperature	On-line Thermocouple	0 – 400° C
ORP	On-line ORP Sensor	±1500 mV ± 1 mV

[4] Test method allows increasing dilutions to obtain a valid enumeration
[5] Incubation Time: 48 hours @ 37° C
[6] BS EN ISO 11731 (2017) documents the number of colonies required on each plate to provide statistical validity to the enumeration process; the range of the number of *Legionella* colonies depends on the presence of a single strain of *Legionella* (10–300 CFU/plate), the presence of interfering microorganisms (10–150 CFU/plate) and the use of a membrane filter technique (10–80 CFU/filter).

Selection of *Legionella* Test Methods

The U. S. Centers for Disease Control (CDC) and most legislation have approved two Legionella test methods to determine an "outbreak:" the standard culture test (7 – 14 days' incubation) and the modified culture test that uses a bacterial enzyme detection technology (7 days' incubation). In February 2021, the CDC added routine testing for *Legionella* by validated test methods, including qPCR.[7] The Public Works and Government Services of Canada[8] requires the use of field qPCR testing to verify the effectiveness of a start-up cleaning and disinfection procedure and during "emergency mode." This document, MD 15161-2013, provides "qPCR Testing Limits and Actions" that have a structure and action levels that are identical to "*Legionella* Culture Testing Limits and Actions." In other words, an action level of 10 CFU/mL of *Legionella* (all sero groups) translates into an action level of 10 UG/mL.

The use of timely Legionella tests is critical because the ecosystem of cooling water is highly dynamic; the population and viability of bacteria can change hourly due to factors such as the heat load and evaporation profile, varying airborne contaminants, degradation of oxidizing biocides by sunlight and concentration of intermittently-fed biocides. Cooling tower owners need rapid field test results to implement timely corrective action and reduce the risk that their cooling tower will discharge Legionella-contaminated water droplets. There are three commercialized technologies for rapid Legionella field tests: an immune-magnetic method (IMM) that detects all sero groups, an antigen-based method, Lateral Flow Immunochromatographic Assay (LFICA), that detects sero group 1, and a genetic method, quantitative Polymerase Chain Reaction (qPCR) that detects all sero groups.

Sensitivity of these test technologies is critical for real-time testing; the CTI guideline defines the performance of the Water Management Plan as "effective" if the Legionella test results are consistently less than 10 CFU/mL. The IMM field test results are qualitative: positive or negative for all Legionella sero groups. The LFICA is a more sophisticated quantitative antigen test that detects the population of *Legionella*, sero group 1 bacteria. The qPCR field test detects the concentration of all sero groups of *Legionella*.

For this study, researchers could have used the IMM field test to identify the end of the inoculation phase (i. e. the beginning of the test phase). Researchers chose a manual field qPCR test during the inoculation phase and the first test phase – Proof of Performance - to obtain information about all sero groups. Researchers contracted with a laboratory holding a CDC Elite qualification to conduct a laboratory culture test for every sample tested using the manual qPCR test. During the second test phase – Sustainable Performance – researchers installed an on-line field qPCR test device that provided daily qPCR test results.

The qPCR test measures the total DNA from live and dead cells, sometimes resulting in higher estimates of *Legionella pneumophila* concentrations than the culture tests that do not detect Viable-But-Not-Culturable (VBNC) bacteria. *Legionella* exists in a VBNC state due to exposure to stressful conditions such as starvation, chemical treatment (e. g. chlorination), too-high or too-low water temperatures, low oxygen concentrations and/or UV treatment.[9] It's likely that the VBNC state is an adaptive strategy that supports long-term survival of the bacteria in unfavorable environmental conditions. It's also likely that, under favorable conditions such as availability of

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[7] "Routine Testing for *Legionella*," Centers for Disease Control (CDC), *Legionella* (Legionnaires' Disease and Pontiac Fever), https://www.cdc.gov/legionella/wmp/control-toolkit/routine-testing.html

[8] MD 15161–2013 Control of *Legionella* in Mechanical Systems, Standard for Building Owners, Design Professionals, and Maintenance Personnel," Issued March 2016, https://www.tpsgc-pwgsc.gc.ca/biens-property/documents/legionella-eng.pdf

[9] Ramamurthy T, Ghosh A, Pazhani GP, Shinoda S. "Current perspectives on viable but non-culturable (VBNC) pathogenic bacteria." Front Public Health. 2014;2:103, https://www.frontiersin.org/articles/10.3389/fpubh.2014.00103/full

nutrients and a host organism (e. g. an amoebae), VBNC cells can survive and reproduce.[10] In other words, the presence of VBNC Legionella bacteria detected by a qPCR test should be treated as a future risk of *Legionella* proliferation when the environmental conditions change. Consequently, researchers relied on data from both field qPCR tests and the laboratory culture to evaluate the efficacy of the PDS technology.

Heterotrophic Aerobic Bacteria. This study used two methods to measure the concentration of heterotrophic aerobic bacteria: a laboratory spread plate culture and a field paddle tester (a. k. a. "dip slide").

Sessile Bacteria. This study used a semi-quantitative method to evaluate the concentration of sessile bacteria.

Legionella. This study used three methods to measure the concentration of *Legionella*: a modified laboratory culture, a manual qPCR field test and an on-line qPCR field test. The modified laboratory culture, approved by the Centers for Disease Control (CDC) for outbreak investigations, results were available approximately seven (7) days after submission to a CDC ELITE laboratory. The manual field qPCR test procedure used pre-measured reagents that included a pH adjustment for "high-conductivity" cooling water; results were available in 45 minutes. The on-line field qPCR test was a completely automated system that allowed researchers to remotely schedule tests and request "on-demand" tests of the cooling water. This system also allows testing of "grab" sample from other sources, including other cooling towers. The test duration is approximately four hours. This on-line field qPCR test device has an onboard PLC to control the test process and the replacement of consumables and uses IoT technology to create a cloud-based data archive.

ATP. This study used a commercially available field test device to measure free and total ATP.

Free Halogen. This study used two methods to measure the concentration of free halogen: a field spectrophotometric method and on-line amperometric sensor.

Copper, Silver, Total Hardness. This study used field spectrophotometric methods to manually measure the concentrations of bromine, copper, silver, and total hardness.

Conductivity. This study used an on-line sensor to measure the conductivity.

Temperature. This study used a thermocouple to measure the temperature of the water entering the PDS.

ORP. This study used an on-line Oxidation-Reduction Potential meter to monitor the oxidizing biocide concentration.

PDS Field Study Design. Before beginning the test, researchers had to wait until the cooling water system had a measurable, sustainable population of Legionella bacteria. This study had two test phases: reduce the population of *Legionella* (i. e. Proof of Performance) and control the bacteria population, including *Legionella* (i. e. Sustainable Control). **Table 2** defines the success criteria for each of these phases.

[10] Casini B, Baggiani A, Totaro M, Mansi A, Costa AL, Aquino F, et al., "Detection of viable but nonculturable *Legionella* in hospital water network following monochloramine disinfection." J Hosp Infect. 2018;9899 (1):46-52.

Table 2 – Bacteria and Biocide – Success Criteria

	Legionella Bacteria (all sero groups)	Heterotrophic Bacteria	Ionization	
	Lab Culture CFU/mL	Lab Culture CFU/mL	[Cu] mg/L	[Ag] mg/L
***Legionella* Growth**	>=100	-	-	-
Proof of Performance	<10	$<10^4$	0.8 – 1.2	0.04 – 0.08
Sustainable Control	Consistently No Growth			

***Legionella* Growth**. Based on the results of the qPCR and lab culture results during the laboratory study, the researchers set a specification for the concentration of Legionella bacteria at or greater than 100 CFU/mL to define the end of the growth phase.

Proof of Performance. This phase of this study continuously operated the non-thermal plasma and varied the operation of the Cu-Ag ionization and oxidizing biocide to study the dose-response of Legionella and heterotrophic aerobic bacteria. Researchers set a specification for the concentration of Legionella bacteria at less than 10 CFU/mL as a criterion of success, consistent with the CTI guideline WTB-148.[11]

Sustainable Control. This phase established continuous operation of the non-thermal plasma and consistent dosing schedules for Cu:Ag ionization and oxidizing biocide to demonstrate sustainable control of bacteria, including *Legionella* and HAB. Researchers set a specification for the concentration of Legionella bacteria to be consistently "no growth" as a criterion of success.

PDS and Cooling Tower #1 - Study Results

***Legionella* Growth.** This cooling tower operates during all seasons because it serves the site's data center. Facilities staff conducted their routine Spring disinfection procedure in April 2021 and cleaned the basin and louvers during the first weekend in June 2021. In early June, researchers idled the plasma and copper-silver ionization treatments to create conditions conducive to the growth of sessile bacteria – where Legionella bacteria reproduce. The PDS system fed oxidizing biocide several times per week to suppress an overgrowth of planktonic, aerobic bacteria that would compete with Legionella bacteria for nutrients. Using field and laboratory test methods, researchers measured the population of heterotrophic and Legionella bacteria every other week. The first evidence of Legionella bacteria in the cooling water occurred on August 16, 2021.

Closer examination of the reasons for the first appearance of *Legionella* indicated that on August 10th, facilities staff reported a high temperature alarm on the cooling water due to an algae bloom. Facilities staff immediately resumed the feed of algaecide. Within several hours, the cooling water temperature decreased. Algaecides control the growth of algae, a plant; these chemicals do not kill bacteria. However, algaecides have surfactant properties. Researchers concluded that the surfactant properties of algaecides can remove some of the sessile bacteria – and the incubating Legionella bacteria in that slime layer.

Following this event, the water treatment service representative resumed the feed of algaecide every Thursday morning at 5 am. **Figure 6** shows that the algaecide consistently released sessile bacteria based on ATP measurements and bacteria tests.

[11] "Legionellosis Guideline: Best Practices for Control of Legionella", CTI Guidelines WTB-148, Cooling Technology Institute, Houston, Texas, https://www.cti.org/downloads/WTP-148.pdf.

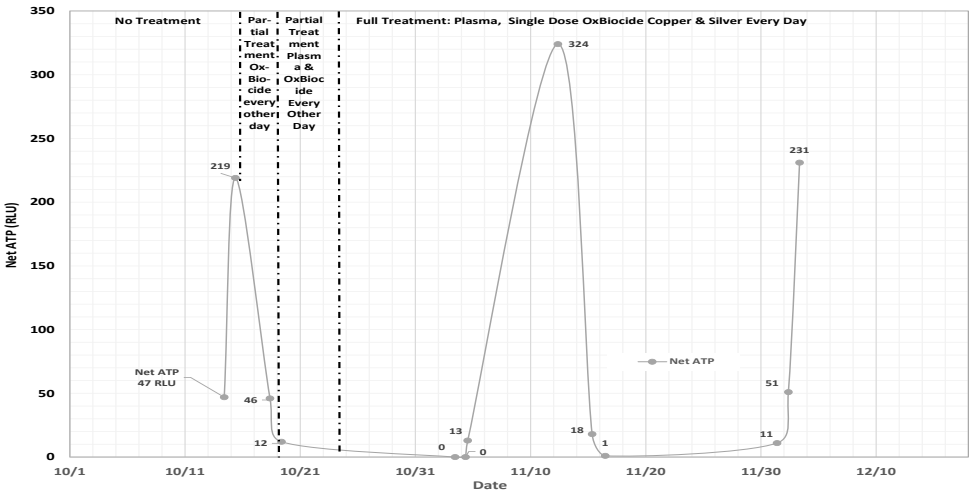

Figure 6 – Effect of Algaecide on Release of Sessile Bacteria

Proof of Performance. The objective of this phase was to ensure that the capacity of the PDS units matched the requirements of a full-scale recirculating cooling water system. Researchers began this phase with no treatment for 11 days to ensure a sustainable population of *Legionella*. Similar to the design of the laboratory study, researchers began the second segment with intermittent copper-silver ionization for five (5) days to demonstrate a reduction in the population of sessile and Legionella bacteria. During the third segment, researchers operated the PDS in the typical mode: continuous plasma, and intermittent feed of Cu-Ag ionization and oxidizing biocide. **Figure 7** shows the performance of the PDS unit for a period of 44 days (August 17 – September 29). The Legionella population decreased from 330 CFU/mL on August 17th to 2 CFU/mL on September 15th – decreasing to non-detectable concentrations on September 28th.

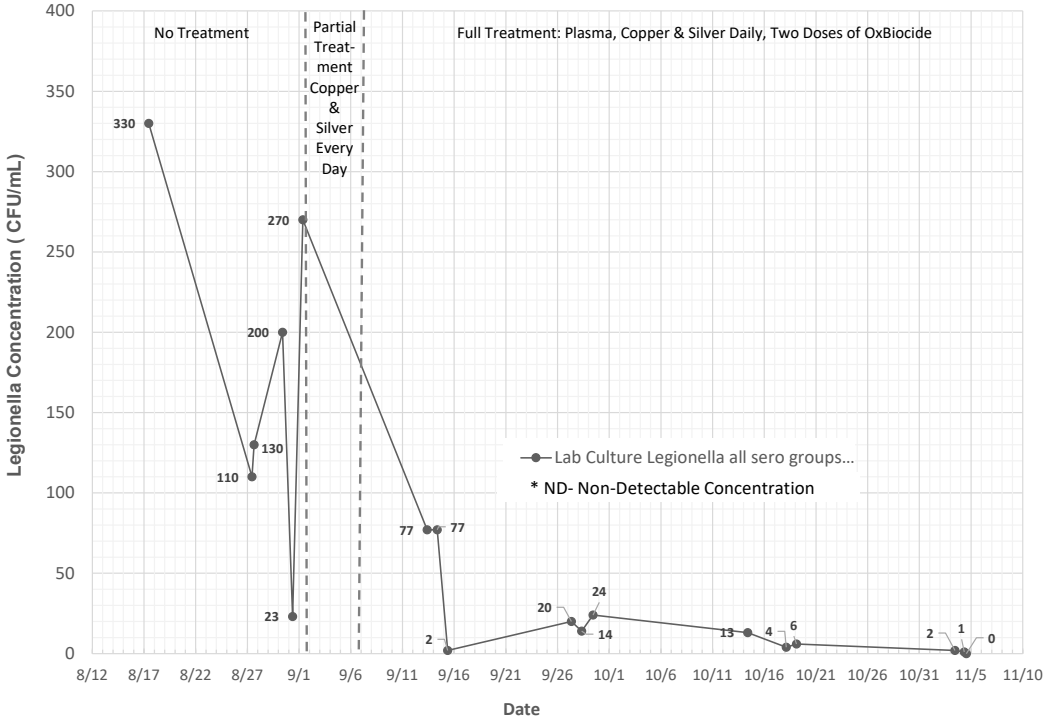

Figure 7 - Proof of Performance: Reduction of Legionella Population (laboratory culture method)

Sustainable Control. The objective of this phase was to demonstrate that the PDS could decrease the concentration of all bacteria and maintain consistent control of the concentration of all bacteria, especially the Legionella bacteria. For this study, researchers installed an on-line, fully-automated Legionella test device that provided frequent qPCR test results. For the first four weeks, researchers obtained on-line qPCR test results several times per week. Starting on November 13, researchers obtained daily qPCR test results (with a few exceptions).

Figure 8 shows the performance of the PDS unit for a period of 65 days (October 16 – December 18). The Legionella population decreased from 265 GU/mL on October 16th to 5 CFU/mL on November 16th. From November 16th through December 18th, the majority of the qPCR test results were at or below 15 GU/ml; however, the results from the laboratory culture tests typically showed "no growth." Researchers hypothesized that the Legionella bacteria might be VBNC, creating a risk of proliferation of Legionella bacteria when environmental conditions were more favorable. A review of the environmental conditions shows temperature water conditions, e. g. daytime water temperatures from (56° F – 72° F) until late November.

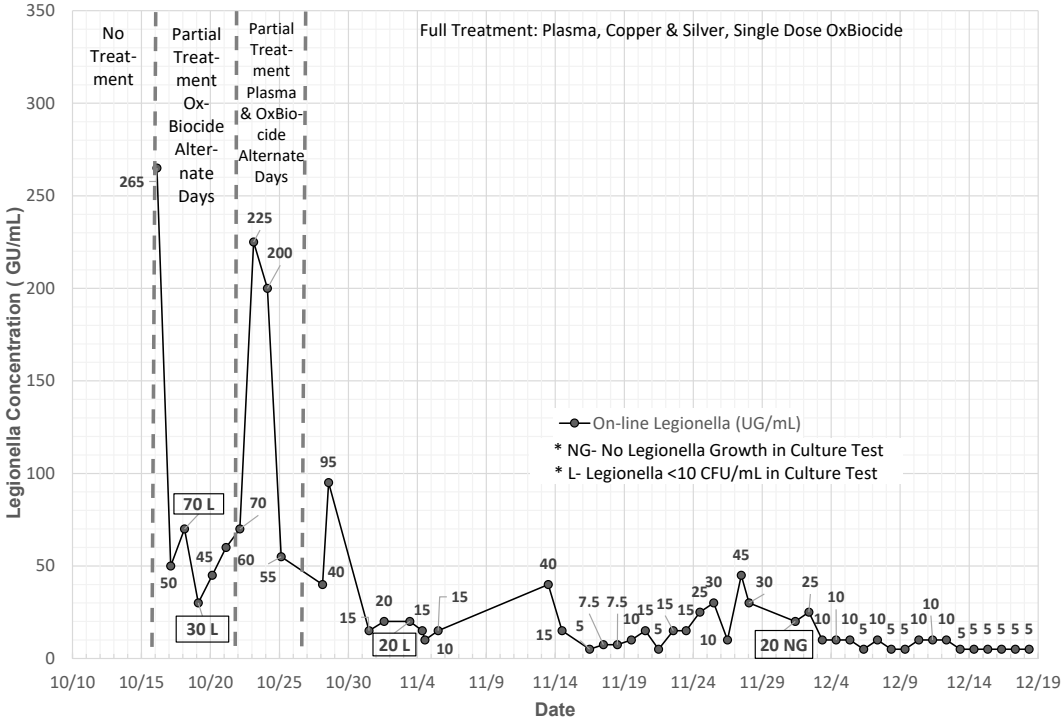

Figure 8 – Sustainable Control: Consistent Low Concentrations of *Legionella* (on-line qPCR method)

Together with the results shown in **Figures 7** and **8**, **Figure 9** confirms that the PDS consistently controlled the population of all bacteria, including aerobic, gram negative and Legionella bacteria.

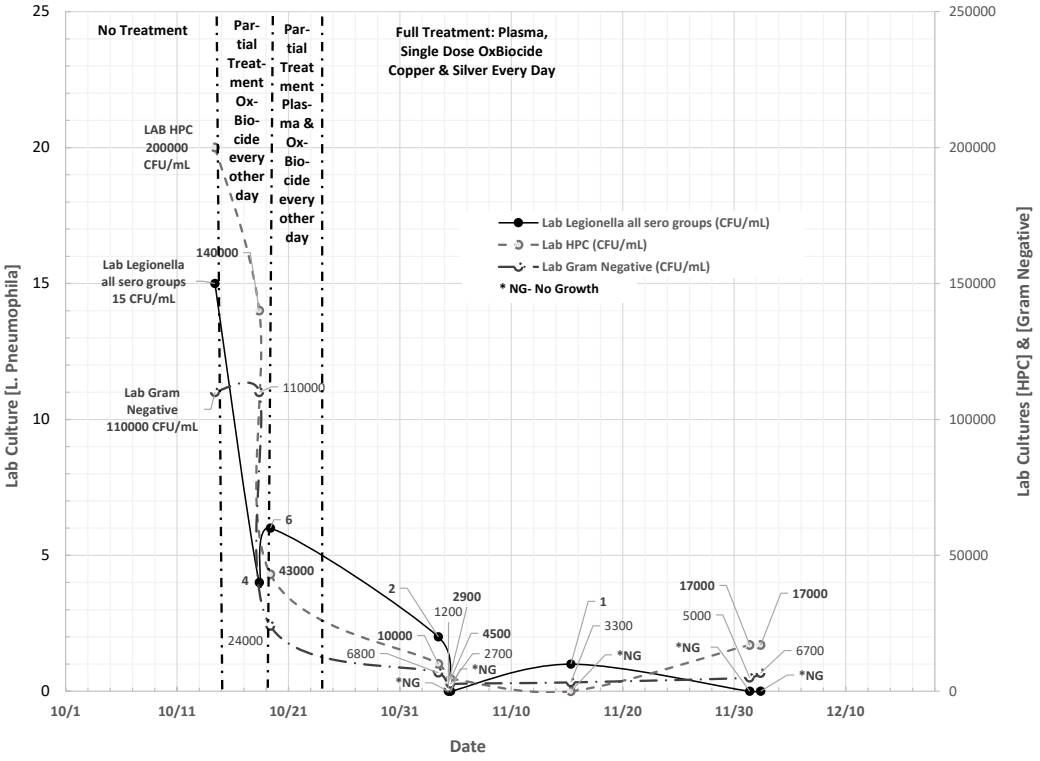

Figure 9 – Sustainable Control: Reduction of All Bacteria Populations (culture methods)

Conclusions

The results of this study confirm the performance of the PDS to sustainably control bacteria populations, including *Legionella*, to safe concentrations. The synergistic efficacy of the novel plasma technology and two traditional technologies (Cu:Ag ionization and oxidizing biocide) kills *Legionella pneumophila* and other gram-negative bacteria and heterotrophic aerobic bacteria in an evaporative cooling water system. The combination of plasma and Cu:Ag ionization is effective in reducing the population of gram-negative bacteria that often exist in a biofilm on water-wetted surfaces in the evaporative cooling water system. The study confirmed the efficacy of algaecide to act as a weak bio-surfactant, releasing sessile bacteria and incubating legionella from surfaces into the bulk water, increasing the efficacy of oxidizing biocides.

The use of the field qPCR test device to obtain real-time measurement of the concentration of *Legionella pneumophila* bacteria allowed researchers to "tune" the PDS unit, adjusting the dosage of each technology to control the populations of sessile and planktonic bacteria.

The use of the automated qPCR test device allows maximum control of legionella populations. Although there is no strict correlation between qPCR and laboratory culture test results, the trend information does support risk management efforts by owners and operators of evaporative cooling water systems. The statistically-small, positive biases in the qPCR test results will prescribe a slightly-higher level of corrective action to reduce the population of Legionella in the recirculating cooling water – a reasonable approach to managing risk.

Researchers are exploring other applications of the real-time Legionella risk management system, including automation of on-line disinfection when recommissioning seasonally-idled cooling towers or during operation in the "shoulder seasons" with low duty cycles that create a low or interrupted flow of recirculating cooling water flowrate, creating a high risk of sessile bacteria proliferation. Providing an effective, flexible bacteria control system and real-time verification of the concentration of *Legionella pneumophila* increases the level of confidence for owners and operators of cooling towers that they are effectively managing their legal and business risks.

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