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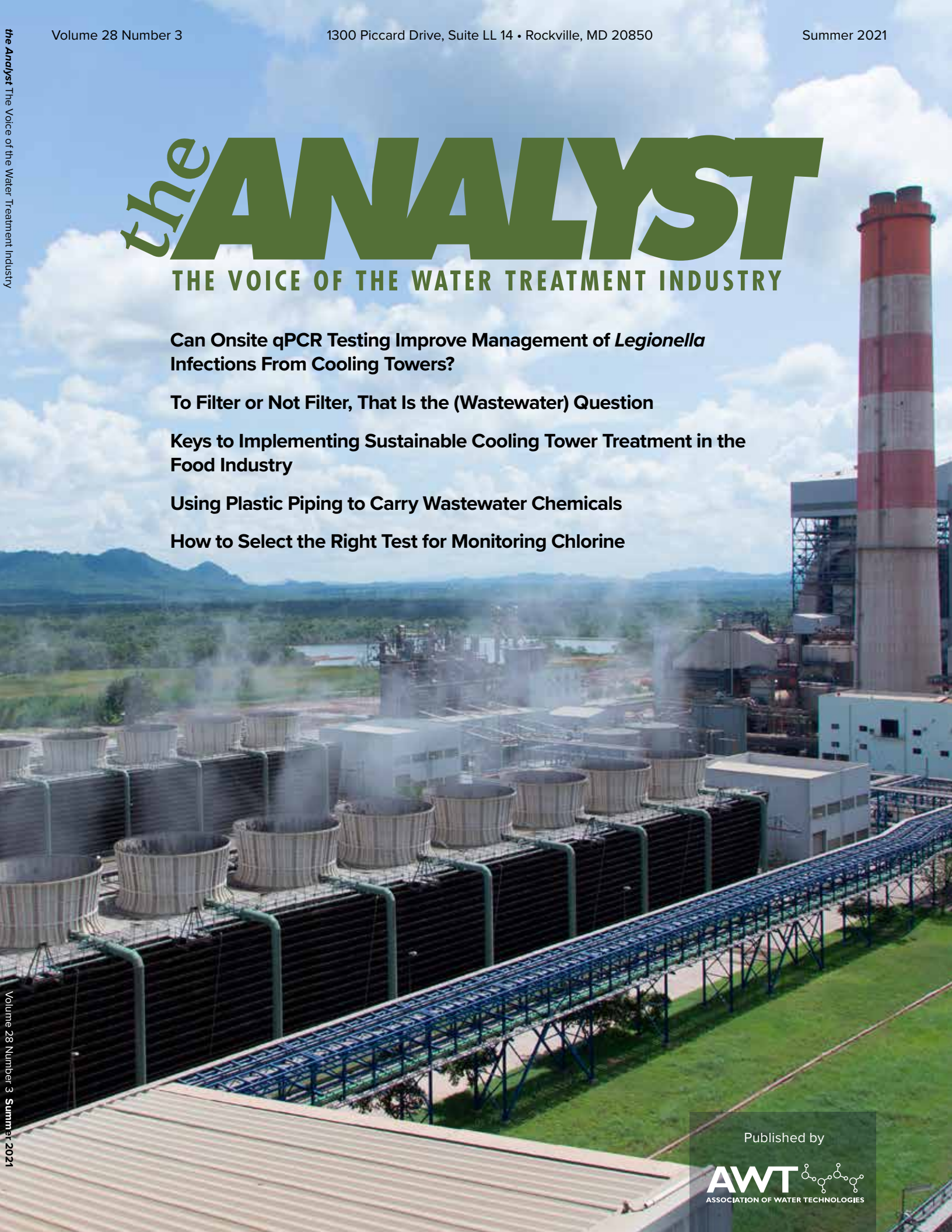

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Can Onsite qPCR Testing Improve Management of *Legionella* Infections From Cooling Towers?

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ASHRAE's Legionellosis risk management standard (1) codifies a systematic process for assessing and controlling the risk of *Legionella pneumophila* in evaporative comfort-cooling water systems. This standard supports a systematic approach to managing bacteria that includes water treatment, routine monitoring, proactive risk assessment, preventative maintenance, and management of water quality during service and idle periods. This article introduces an innovative plasma disinfection system (PDS) that provides biocidal treatment to control 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 nonoxidizing biocides.

The key benefits from controlling the population of sessile bacteria and the associated biofilm are improving the heat transfer (2), reducing the rate of proliferation of *Legionella* (3), and minimizing the adaptive ability of bacteria (4) by using multiple biocidal technologies (5). By using a novel, sophisticated onsite *Legionella* qPCR test device, owners of cooling towers can quickly measure the *Legionella* population and proactively optimize bacteria control by making real-time adjustments in the PDS.

This article describes the use of an onsite *Legionella* qPCR rapid-test device and an innovative bacterial control system to control the population of *Legionella* bacteria in a model evaporative cooling water system. The sample-to-result turnaround time is less than one hour, allowing for immediate adjustments to the PDS. Researchers documented the value of the qPCR test results for assessing the risk of *Legionella* bacteria by comparing the qPCR test results to the results of a modified culture test and confirmed the efficacy of the innovative bacterial control system to control planktonic and sessile bacteria.

Plasma Disinfection System

The patented PDS (6) pairs two traditional technologies—copper-silver biocidal ions and hypobromous acid—with nonthermal atmospheric plasma, a novel technology. The PDS controls sessile and planktonic bacteria, including *Legionella* and heterotrophic aerobic bacteria (HAB).

"The PDS controls sessile and planktonic bacteria, including *Legionella* and heterotrophic aerobic bacteria (HAB)."

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 brief period of electrical discharge, the elevated 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 very 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 and silver ionization. This system includes sacrificial electrodes to generate and automatically dose copper and silver ions to control sessile bacteria.

Oxidizing biocides. The electrolyzer produces sodium hypochlorite from a brine solution. An automatic feedback control system uses an online oxidation-reduction potential (ORP) sensor for feedback control of a mixture of sodium hypochlorite and hypobromous acid to control planktonic bacteria. The PDS system includes an online 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 and probe temperature] fan speed, pump speed, inlet and outlet water pressure, water flow rate, inlet and outlet water temperatures, leak sensor) and water quality parameters from online sensors (conductivity, pH, ORP, temperature).

Figures 1 and 2 show the configuration of the prototype unit evaluated in this laboratory study and now installed at a corporate campus supporting the first field study—two parallel evaporative cooling water systems: a "test" system using the innovative PDS technology and a "control" system using conventional oxidizing and non-oxidizing biocides.

Figure 1: PDS user interface.

Figure 2: Plasma/copper-silver ionization unit and oxidizing biocide generator.

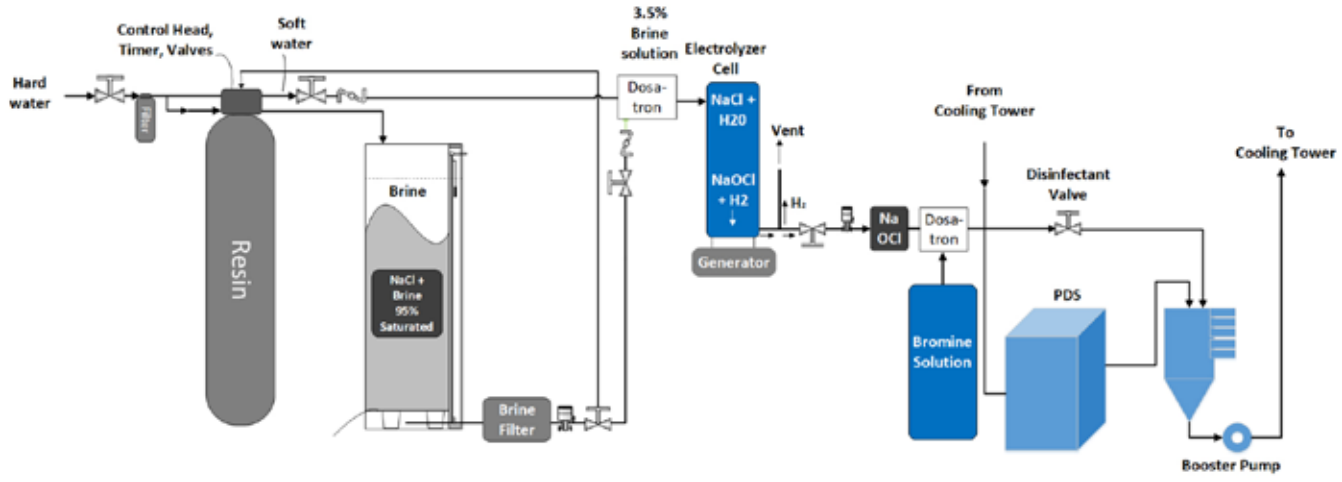
Figure 3 shows the process flow; a portion of the recirculating cooling water is treated by the PDS unit and returns to the recirculating cooling water system. A second skid generates and feeds the oxidizing biocide: a bromine storage tank and an electrolyzer that generates sodium hypochlorite. This system uses Internet of Things (IoT) technology to create a cloud-based data archive.

Legionella Test Methods

The Centers for Disease Control (CDC) and New York City and New York State regulations and legislation have approved two *Legionella* test methods to determine an "outbreak": the standard culture test (7–14 day incubation) and the modified culture test, which uses a bacterial enzyme detection technology (7-day incubation). These laboratory tests are a poor fit for cooling tower owners because the objective of routine monitoring is not to identify an outbreak of Legionnaire's disease but rather to provide a timely indication of the viability of *Legionella* bacteria in the recirculating cooling water, allowing owners to proactively implement bacteria control procedures to minimize the risk of legionellosis infections.

Timely *Legionella* test results are 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.

Figure 3: Process flow diagram—PDS and electrolyzer system.



There are two commercialized technologies for *Legionella* field tests: immune-magnetic method (IMM) and quantitative polymerase chain reaction (qPCR). The sensitivity of these technologies is critical for real-time testing; the Cooling Technology Institute (CTI) guideline defines the performance of the Water Management Plan as "effective" if the *Legionella* test results are consistently less than 10 colony-forming units per milliliter (CFU/mL) (7). The IMM field test that measures tagged antibodies lacks sufficient sensitivity (minimum detection limit: 100 CFU/mL). The qPCR field test has sufficient sensitivity to serve as an early warning of *Legionella pneumophila* proliferation. The minimum detection limit is 8 CFU/mL, which is an approximate correlation from genomic units (GU). The analysis time for qPCR field tests ranges from one to four hours.

Although studies have not shown a reproducible correlation between the qPCR test results and the culture results, the field qPCR test results provide relevant information for routine monitoring and control of the risk of *Legionella pneumophila* bacteria (8). Because the qPCR measures DNA, this test has several advantages over the culture methods, including specificity, no cross-reactivity with other bacteria, and the ability to identify true negative results and sensitivity. The test also shows the ability to identify true positives by detecting low concentrations of DNA. The fact that qPCR measures the total DNA from live and dead cells sometimes results in higher estimates of *Legionella pneumophila* concentrations than the culture tests that do not detect viable-but-not-culturable (VBNC) bacteria. The qPCR test does not provide any serotype information. In rare cases, the qPCR test may react with non-*Legionella pneumophila* bacteria, creating a false positive. These statistically small, positive biases in the qPCR test results will prescribe a slightly higher level of corrective action to reduce the population of *Legionella pneumophila* in the recirculating cooling water—a reasonable approach to managing risk.

This model cooling tower study used both the qPCR test method and the modified culture test method to validate the value of the field test to assess and manage the risk of *Legionella pneumophila* in evaporative cooling water systems.

Discussion

PDS Test System. The PDS system used in this test is a full-scale pre-production model. Key specifications include the electrical requirements [208 V, single phase, 20 A, 50/60 Hz]; nominal energy requirements [1,000 KVA]; ambient conditions [conditioned space < 40 °C (105 °F)]; cooling water sample flow rate (20 gallons per minute [gpm]); dimensions [81.5 centimeter [cm] x 61 cm x 152.5 cm (32" x 24" x 60")]; and weight [340 kg (750 pounds [lb])]. A second skid, integrated with the PDS PLC via Modbus communications, includes a bromine storage tank and the electrolyzer equipment [181 kg (400 lb.), 145 cm X 94 cm X 183 cm (45" X 37" X 72")].

Model Cooling Tower. Researchers conducted this test in a Baltimore Aircoil (BAC) cooling tower rated for 12 tons installed behind the research facility. A gas-fired heater produced water at approximately 30 °C (85 °F) to simulate the normal operation of a recirculating evaporative cooling water system. This test did not include the addition of traditional water treatment chemicals for deposit and corrosion control.

Test Methods and Analytical Measurements. Table A 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 incredibly low concentrations.

"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."

Table A: Analytical Measurements

Measurement	*Equipment*	*Test Method Range*
Heterotrophic Aerobic Bacteria	Laboratory spread plate culture	--
Heterotrophic Aerobic Bacteria[1]	Field paddle tester	10^2–10^6 CFU/mL
Gram-Negative Bacteria	Field paddle tester	--
Legionella	Laboratory modified culture	--
Legionella	Field qPCR test	1–80,000 GU/mL
Free Halogen	Colorimeter	0.04–4.00 mg/L
Halogen, pH	Online amperometric free halogen analyzer	0.04–4.00 mg/L, 0–14 pH
Bromine	Spectrophotometer	0.04–10 mg/L
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	Online conductivity sensor	10–10,000 mS/cm
Temperature	Online thermocouple	0–400 °C
ORP	Online ORP sensor	±1,500 mV ± 1 mV

Research Approach

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.

Gram-negative bacteria: This study included the measurement of gram-negative bacteria using the field paddle tester. *Legionella* species, as well as other pathogens such as *Escherichia coli* (*E. coli*) are gram-negative bacilli that have a thick cell wall and outer layer and often exist within biofilms. For this study, the incubation time was 48 hours at 37 ˚C.

***Legionella*:** This study used two methods to measure the concentration of *Legionella*—modified laboratory culture and qPCR field test. The modified laboratory culture, approved by the CDC for outbreak investigations, provides results approximately seven days after submission to the laboratory. The qPCR test procedure used pre-measured reagents that included a pH adjustment for "high-conductivity" cooling water had available results in 45 minutes.

Free Halogen: This study used two methods to measure the concentration of free halogen—manual colorimetric method and online amperometric sensor.

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

Conductivity: This study uses an online sensor to measure the conductivity.

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

ORP: This study uses an online oxidation-reduction potential meter to monitor the oxidizing biocide concentration.

PDS and Model Cooling Tower—Study Design

The study has two parts: inoculation and test. The objective of this study was to demonstrate the efficacy of several combinations of biocidal modalities. Tables B and C define the water quality specifications and success criteria.

Table B: Water Quality—Specifications

Phase	*Water Temp (˚C [˚F])*	*pH*	*Conductivity (mS/cm)*	*[Calcium Hardness] (mg/L as $CaCO_3$)*	*ORP (mV)*	*Free Halogen (mg/L)*
Inoculation	27–32 (80–90)	8.4–8.8	NS	NS	0	0
Test	27–32 (80–90)	8.4–8.8	NS	NS	NS	0.2–1.2
NS = not specified						

Table C: Bacteria and Biocide—Success Criteria

	Legionella Bacteria		*Heterotrophic Bacteria*			*Ionization*	
Phase	*qPCR (GU/mL)*	*Modified Culture (CFU/mL)*	*HAB (CFU/mL)*	*HPC (CFU/mL)*	*Gram-Negative (CFU/mL)*	*[Cu] (mg/L)*	*[Ag] (mg/L)*
Inoculation	>1,000	>150	NS	NS	NS	0	0
Test	ND	<10	<1X104	<1X104	NS	0.8–1.2	0.04–0.08
ND = non-detectable NS = not specified							

This study used potable water supplied by the municipal water authority, Loudoun Water. The low concentrations of free halogen (0.08 mg/L) in the potable water did not impact the results of this study. The relatively high concentrations of copper (0.12 to 0.21 mg/L) in the potable water at the laboratory may have influenced the results of the test phase.

Inoculation phase. Although there is no strict correlation between the results of the qPCR and the laboratory culture, the researchers' used their experience with this model cooling tower to define the end of the inoculation period: >1,000 GU/mL for 24 hours that results in a *Legionella* concentration above 1,000 CFU/mL.

Test phase. The test phase of this study sequenced each of the three modes in the following manner: plasma only ~three-day segment; plasma and copper:silver (Cu:Ag) ionization ~two-day segment; plasma, Cu:Ag ionization and oxidizing biocide ~two-day segment. The performance of each mode depends on several factors: duration of the test segment; dosage of copper, silver, and bromine; and the concentration of *Legionella* and heterotrophic aerobic bacteria. The test conditions in the third phase, plasma + Cu:Ag ionization + oxidizing biocides, most closely matches the environmental conditions in an evaporative cooling water system. All specification limits are consistent with application guidelines for evaporative cooling water systems.

PDS and Model Cooling Tower—Study Results

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. Water treatment chemicals serve as nutrients for bacteria. Researchers compensated for the absence of water treatment chemicals by creating a concentration of *L. pneumophila* (1,300 CFU/mL) that was an order of magnitude higher than the CTI Guideline WTP-48 (9) for online disinfection ("hyper-halogenation") during the six-day inoculation phase.

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). Table D defines the water quality during the test.

Table D: Water Quality—Specification Limits and Test Results

End of Each Phase	*Water Temp (˚C [˚F])*	*pH*	*Conductivity (mS/cm)*	*Calcium Hardness (mg/L as $CaCO^3$)*	*ORP (mV)*	*Free Halogen (mg/L)*
Inoculation	30.4 (86.7)	8.80	1,179	658	176	<0.02
Plasma-only	31.8 (89.2)	8.81	1,236	700	140	<0.02
Plasma + Cu:Ag ionization	30.8 (87.5)	8.81	1,233	660	142	<0.02
Plasma + Cu:Ag Ionization + Oxidizing Biocides	31.4 (88.5)	8.61	1,264	656	594	1.14
Test Phase Specs	27–32 (80–90)	8.4–8.8	NS	NS	NS	0.1–1.2

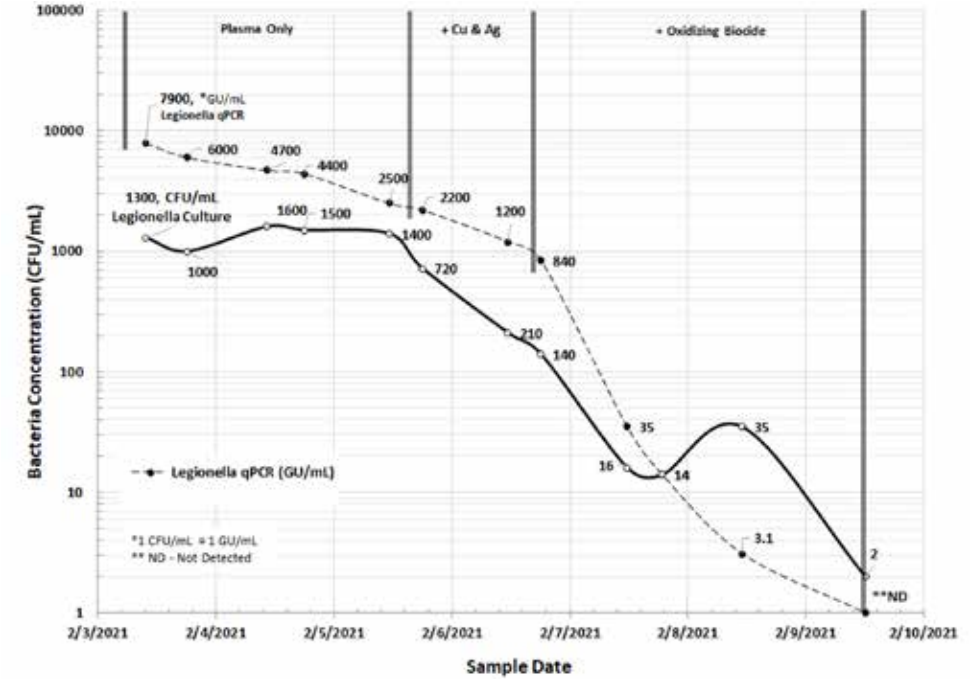
Table E defines the success criteria and summarizes the test results for each phase.

Table E: Bacteria and Biocide—Success Criteria and Test Results

	Legionella Bacteria		*Heterotrophic Bacteria*			*Ionization*	
Test Phase (Duration)	*qPCR (GU/mL)*	*Modified Culture (CFU/mL)*	*HAB (CFU/mL)*	*HPC (CFU/mL)*	*Gram-Negative (CFU/mL)*	*[Cu] (mg/L)*	*[Ag] (mg/L)*
Inoculation (six days)	7,900	1,300	1X106	16,000	3,600	0.11	0
Plasma-only (56 hours)	2,200	720	100	940	410	0.21	0
Plasma + Cu:Ag Ionization (24 hours)	840	140	<100	360	NG	0.09	0.07
Plasma + Cu:Ag Ionization + Oxidizing Biocides (56 hours)	ND	2	ND	10	NG	0.06	0.06
Success Criteria	ND	<10	<1X104	<1X104	NS	0.8–1.2	0.04–0.08
NG = no growth NS = not specified ND = non-detectable							

Although there is no strict correlation between the results of the *Legionella* culture and the qPCR tests, the trend information shown in Figure 4 confirms that having real-time information about the concentration of *L. pneumophila* bacteria allows proactive corrective action.

Figure 4: *Legionella*-modified culture (CFU/mL) versus qPCR (GU/mL) in a model cooling tower (February 2021).



Although the field paddle test method is a semi-quantitative method, with no sensitivity below 100 CFU/ml, Figure 5 shows that the trend information is consistent with laboratory spread plate culture method.

Figure 5: Heterotrophic culture versus paddle tester aerobic bacteria (CFU/mL) in a model cooling tower (February 2021).

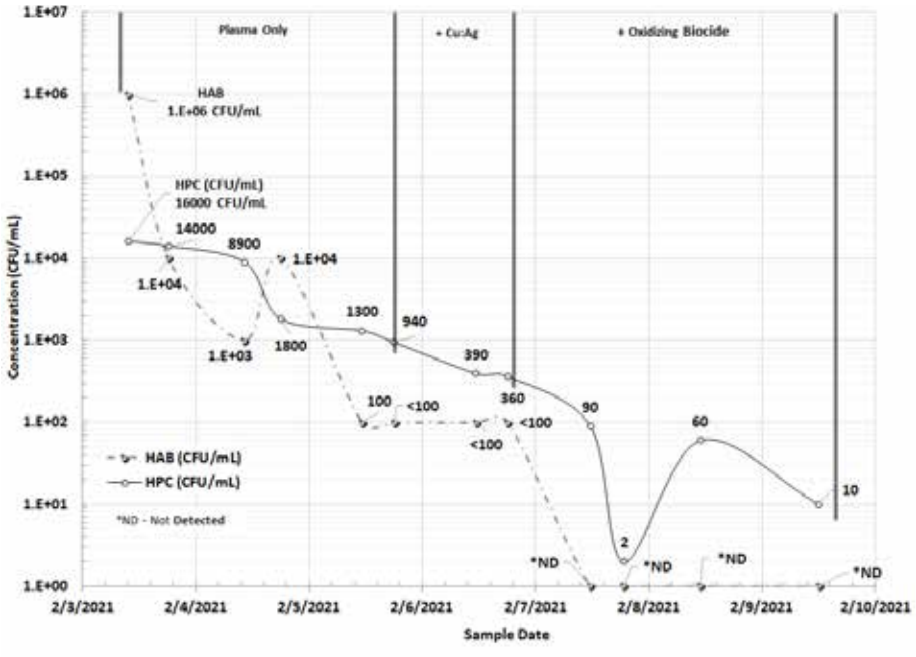


Figure 6 shows that the information provided by the field paddle test method for gram-negative bacteria provides useful information about the efficacy of the nonthermal plasma and copper: silver (Cu:Ag) ionization technology to control sessile bacteria.

Figure 6: *Legionella*-modified culture (CFU/mL) versus qPCR (GU/mL) and gram-negative bacteria (CFU/mL) in a model cooling tower (February 2021).

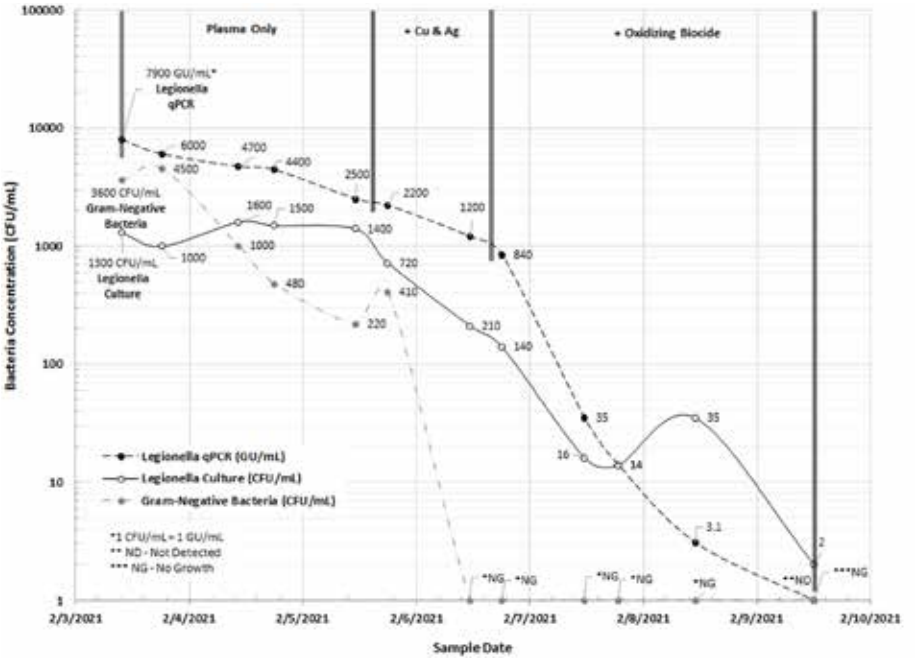
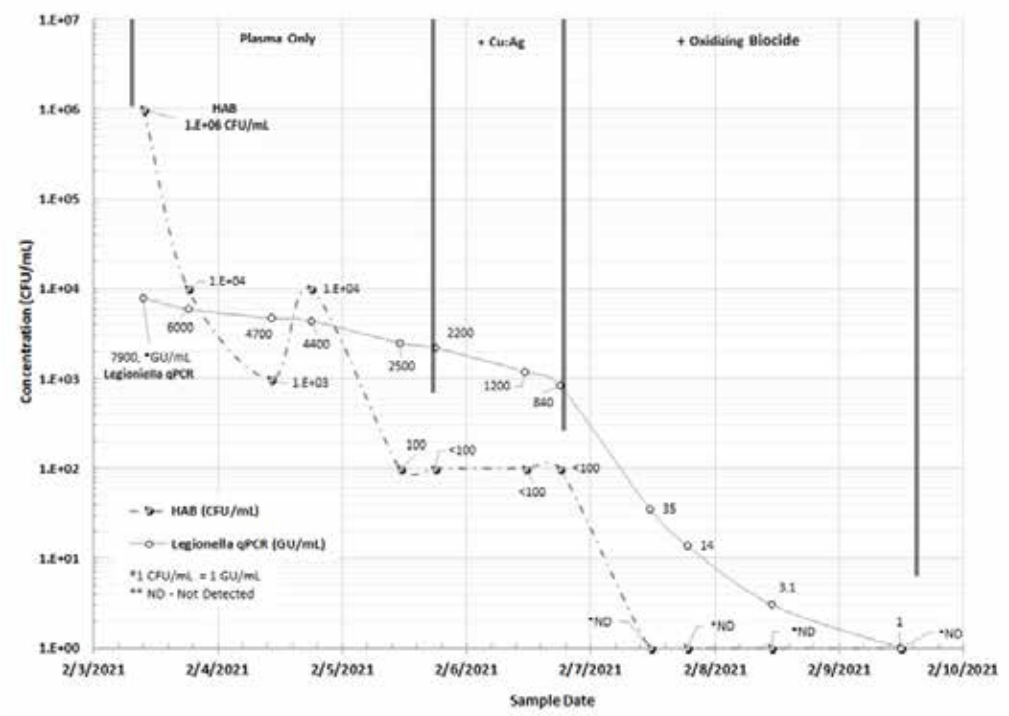


Figure 7 shows the bacteria data available to an owner or operator of an evaporative cooling tower using the PDS and the field qPCR *L. pneumophila* test to control bacteria.

Figure 7: *Legionella* qPCR (GU/mL) and Paddle Tester Aerobic Bacteria (CFU/mL) in a Model Cooling Tower (February 2021).



Conclusions

The results of this study confirm the synergistic efficacy of the novel plasma technology and two traditional technologies (Cu:Ag ionization and oxidizing biocide) to kill *L. 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 suitability of the field paddle test method to provide information about the concentration of aerobic and gram-negative bacteria.

The use of the field qPCR test device to obtain real-time measurement of the concentration of *L. pneumophila* bacteria allowed researchers to "tune" the PDS unit, adjusting the dosage of each technology to control the populations of sessile and planktonic bacteria. 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 planning the next stage of investigation to demonstrate consistent control of *Legionella* bacteria in a field environment. This first field trial at a corporate location has two parallel, separate, recirculating cooling water systems partnered with two similarly sized cooling towers. This field trial will install the PDS on one evaporative cooling water system. The other evaporative cooling water system will use a conventional biocide treatment program and serve as a control for the investigation. The water treatment program for deposit and corrosion control will be identical for both towers: a conventional chemical treatment program with dispersants and anodic and cathodic corrosion control products. Researchers will evaluate bacteria control in both cooling water systems during the cooling season.

Researchers are exploring other applications of the real-time *Legionella* risk management system, including

automation of online disinfection when recommissioning seasonally idled cooling towers or during operation in the "shoulder seasons" with low-duty cycles that create low or interrupted flow of recirculating cooling water flow rate, creating an elevated 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.

Acknowledgements

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