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AWWA WATER SCIENCE

ORIGINAL RESEARCH

Monitoring distribution systems for *Legionella pneumophila* using Legiolert

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This study implemented the Legiolert test (a culture-based assay for *L. pneumophila* based on the most probable number [MPN]) at 12 utilities to assess their experiences and to develop a baseline of *Legionella pneumophila* occurrence in drinking water distribution systems. A total of 679 samples were analyzed during the study: 53 source water, 50 from the plant effluent, and 576 from the distribution system. *L. pneumophila* was detected in three of five source water samples at one utility but was not detected in any of the treated plant effluent samples. *L. pneumophila* was detected in only one distribution sample (0.17%) at a concentration of 1 MPN/100 mL in a sample that contained 0.72 mg/L free chlorine and was serotyped as belonging to the 2–14 serogroup. Four (0.7%) distribution samples could not be confirmed by serotyping. Overall, the utilities found the test easy to learn and apply in their systems. This study provides a precedent for future monitoring of drinking water systems.

KEYWORDS
distribution, *L. pneumophila*, Legiolert, *Legionella*, microbiology

1 | INTRODUCTION

Legionellosis is a respiratory infection caused by bacteria in the genus *Legionella.* Currently, there are approximately 50 species of *Legionella* consisting of 70 serogroups, but *Legionella pneumophila* serogroup 1 is responsible for about 95% of the Legionnaires' disease cases in the United States (Fields, Benson, & Besser, 2002). The incidence of Legionnaire's disease has increased over 550% since 2000 (Figure 1). It is not clear whether the increase is due to increased awareness and testing, an aging population with increased susceptibility to the disease, increased *Legionella* in the environment, or some combination of factors. Water is the natural reservoir for *Legionellae*, and the bacteria are found worldwide in many different natural and manmade aquatic environments, such as cooling towers; water systems in hotels, homes, ships, and factories; respiratory therapy equipment; fountains; misting devices; and spa pools (Fields et al., 2002).

L. pneumophila has become the most commonly identified drinking water pathogen, responsible for about two-thirds of all potable water outbreaks and nearly all the fatalities associated with drinking water outbreaks since 2000 (Benedict et al., 2017). Although outbreaks typically occur in building water systems and cooling towers, potable water utilities typically do not monitor for this important pathogen. *Legionella* is regulated under the Surface Water Treatment Rule (SWTR) with a maximum contaminant level goal (a nonenforceable guideline) of zero *Legionella* organisms for drinking water (U.S. Environmental Protection Agency [USEPA], 1989). The rule specifies a treatment technique for *Legionella* control (e.g., filtration and maintenance of a detectable disinfectant residual), and therefore, monitoring for *Legionella* is not required. Although analytical methods existed for *Legionella* detection, the USEPA determined that testing was impractical to implement, particularly for small systems.

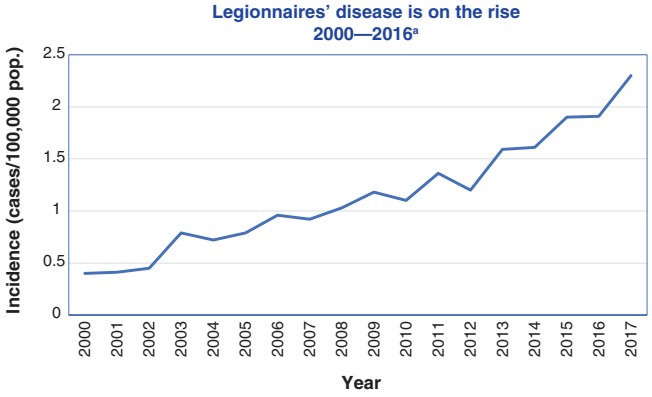

FIGURE 1 Increase in Legionnaires' disease in the United States. *Source*: Centers for Disease Control and Prevention. [a]National Notifiable Disease Surveillance System

Legiolert (IDEXX Laboratories Inc., Westbrook, ME) is a culture-based assay for *L. pneumophila* based on the most probable number (MPN) and similar to the Colilert test. Sartory, Spies, Lange, Schneider, and Langer (2017) reported that the Legiolert test resulted in significantly higher counts of *L. pneumophila* than the standard International Organization for Standardization (ISO) 11731-2 membrane filtration method based on a multilaboratory study. They examined 290 paired counts for both the ISO and Legiolert methods, with a mean of 132 MPN/100 mL for the Legiolert test (range 0–2,273 MPN/100 mL) compared with a mean of 26 cfu/100 mL for the ISO 11731-2 method (range 0–368 cfu/100 mL). Of the 1,105 isolates examined, 96.7% were confirmed by serology to be *L. pneumophila* (Sartory et al., 2017). The superior performance of the Legiolert method was attributed to the broth incubation (rather than membrane filtration) and the extended counting range of the Quanti-Tray format. Spies et al. (2018) reported data from a multilaboratory study that showed the 100 mL Legiolert MPN method was equivalent to the highest result of either ISO 11731 or ISO 11731-2 regardless of whether nonpneumophila species of *Legionella* were included in the evaluation. The Legiolert method had a specificity for *L. pneumophila* of 97.9%, comparable to the 95.3% specificity for the ISO 11731 method. The authors concluded that the Legiolert method represented a significant improvement in the enumeration of *L. pneumophila* from drinking water and related samples. Petrisek and Hall (2018) compared the Legiolert test with method 9,260 J (*Standard Methods*, 2017) for the enumeration of *L. pneumophila* from potable and nonpotable waters and reported that Legiolert exhibited higher sensitivity for the detection of *L. pneumophila* for potable water and equivalent sensitivity for nonpotable water. For the Legiolert potable water samples, they reported a false positivity value of <0.5% and a specificity of 100%. Similarly, for the nonpotable water analysis, Legiolert had a false positivity value of <0.9% and a specificity of 100%. Similarly, Rech, Swalla, and Dobranic (2018) reported increased sensitivity, low false positive rates, and less interference for nonpotable water cooling tower samples than the Centers for Disease Control and Prevention (CDC) culture method.

The objective of this study was primarily to gain U.S. utility experience with the Legiolert method and evaluate the training and learning curve of the test for both potable and nonpotable water samples. Testing was conducted by 12 water utilities to collect data on the occurrence of *L. pneumophila* in distribution systems. The study organizers worked with one state agency to develop guidelines for responding to positive potable water *Legionella* samples. Although this was a small study, it sets important precedents for future monitoring of drinking water systems.

2 | MATERIALS AND METHODS

2.1 | Utility recruitment

A total of 45 water utilities initially expressed interest in participating in the study. Details of the study, the number of samples to be collected, a description of the analytical procedures, and an anticipated quality assurance and quality control (QA/QC) training was provided (see Appendix S1, Supporting Information). In addition, each candidate was provided with a data collection form and a draft memo that could be used to discuss the project with state regulators. Many of the prospective utilities expressed concerns about the possibility of detecting *L. pneumophila* in their distribution system, and at least one utility was told by their state regulator that a "do not use" order would be issued if they detected *L. pneumophila* in their distribution system. That utility subsequently declined to participate. Other utilities expressed concern about the laboratory workload or that other projects were currently underway. In the end, 12 utilities agreed to participate and represented a good geographic distribution, with five sites along the East coast and four in the West as well as three sites within the center of the United States. Sites were also distributed equally, with six sites both in the northern and southern parts of the United States. Table 1 shows the characteristics of the systems. Ten of the systems used surface water, one used groundwater that was recharged with surface water, and the other had a blend of surface water and groundwater. Two systems were unfiltered, one used ultramembrane filtration, and the others used various forms of granular media filtration. For primary disinfection, two systems used ozone and ultraviolet (UV) disinfection, one used UV alone, two used chlorine dioxide, six used prechlorination, and one used prechloramination with sufficient contact time to meet the SWTR requirements (USEPA, 1989). Five of the systems maintained a chloramine residual in the distribution system, while the other seven sites used free chlorine.

2.2 | Sampling sites

The study plan included collecting four raw water, four plant effluent, and approximately 48 distribution system samples from each utility. Some systems collected additional samples

TABLE 1 Characteristics of participating systems

Utility code	Population served	Water source	Filtration	Primary disinfectant	Secondary disinfectant	Miles of pipe
1	50,000	Surface	Unfiltered	Ozone and UV	Chloramine	1,000
2	8,500,000	Surface	Unfiltered	UV	Free chlorine	6,500
3	1,600,000	Surface	Filtered	Free chlorine	Chloramine	3,200
4	950,000	Surface	Sand/GAC filtered	Free chlorine	Free chlorine	4,200
5	750,000	Surface	GAC/anthracite/sand filtration	Chlorine dioxide/chlorine	Free chlorine	200[a]
6	450,000	Surface	GAC filtration	Free chlorine	Free chlorine	2,300
7	500,000	Surface	Anthracite/sand filtration	Chloramine	Chloramine	1,600
8	725,000	Ground	—	Free chlorine	Free chlorine	4,600
9	2,100,000	Surface/ground	Multistage filtration	Ozone and UV	Free chlorine	6,500
10	65,000	Surface	Direct filtration, anthracite/sand filtration	Chlorine dioxide	Chloramine	600
11	90,000	Surface	Anthracite/sand filtration	Free chlorine	Free chlorine	410
12	500,000	Surface	Ultrafiltration	Free chlorine	Chloramine	1,000

Note. GAC: granular activated carbon; UV: ultraviolet.
[a] Regional wholesale system services multiple consecutive systems.

or had multiple treatment plants. The participating utilities were instructed to develop a sampling plan for each system that represented a cross section of the distribution. Samples were to be collected from sites with a known history of water quality results. Most systems used their existing Total Coliform Rule (TCR) monitoring locations, but 26 (4.5%) of the 576 distribution system samples were from finished water reservoirs or storage tanks. Some of the utilities had coded sampling locations, so it is possible that additional samples were from storage facilities. Samples were collected starting in November 2017 through April 2018.

2.3 | Analytical methods

Table 2 shows a summary of the analytical methods used in this study. All supplies used for the microbiological analyses were provided at no cost by the manufacturer so that all the utilities collected data using the same methodology. All analyses were conducted according to the manufacturer's protocols or according to *Standard Methods* (2017).

Briefly, the Legiolert method consisted of collecting a 100-mL potable water sample and allowing the temperature to equilibrate to room temperature. The water hardness was adjusted, if necessary, using reagents supplied in the kit. The Legiolert reagent was added to the sample, shaken, and poured into a Quanti-Tray/Legiolert and was sealed using a Quanti-Tray Sealer PLUS (IDEXX Laboratories Inc.). The trays were incubated at 39 ± 0.5°C for 7 days and examined for production of a brown color and/or microbial growth (as evidenced by turbidity) relative to a negative control. The number of tray wells positive was counted, and an MPN/100 mL was calculated using a formula provided in an Excel spreadsheet. All positive samples were sent to the IDEXX reference lab for serotyping using a latex agglutination kit (Oxoid; Thermo Fisher Scientific, Fremont, CA).

Nonpotable water samples were processed similarly, except 2.0 mL of source water was mixed with 2.0 mL of Legiolert pretreatment reagent for 60 s (±5 s), and then, 2.0 mL of the solution was added to 100 mL of sterile diluent (consisting of deionized water, dechlorinated water, phosphate buffer, Butterfield's buffer, or 0.1% peptone) to which the Legiolert reagent had been added. The solution was incubated in the Quanti-Tray/Legiolert at 37 ± 0.5°C for 7 days.

TABLE 2 Summary of analytical methods

Parameters to test	Test performed
Legionella pneumophila (MPN/mL)	Legiolert
Total coliform/*Escherichia coli* (presence/absence)	Colilert
HPC (cfu/mL)	SimPlate for HPC
Free/total chlorine	DPD or amperometric titration method
Temperature	Thermometer
pH	pH strip or electrode
Total organic carbon	SM5310B

Note. DPD: *N,N*-diethyl-*p*-phenylenediamine; HPC: heterotrophic plate count; MPN: most probable number.

2.4 | Training QA/QC

Most of the participating utilities had a representative from the manufacturer visit their laboratory and conduct a short training program. During the training, a positive control, negative control, and sterile blank samples were prepared and examined. The positive control consisted of the IDEXX-QC *L. pneumophila,* and the "nontarget" negative control was IDEXX-QC *Enterococcus faecalis.* In addition, source water and distribution system samples were processed as part of the training session.

It is important to maintain adequate humidity during the 7-day incubation to avoid moisture loss within the Quanti-Tray/Legiolert. As a QA/QC procedure, laboratories were instructed to monitor the weight of the Quanti-Tray/Legiolert before and after the 7-day incubation and verify that not more than 15% weight loss occurred during incubation. If the postincubation

trays were <85% of their original weight, the laboratories were instructed to consult the manufacturer for further guidance.

2.5 | Poststudy survey

An online survey was sent to all participants after all the monitoring was completed. The results were collected anonymously, and the participants were encouraged to be frank in their assessment of the study. Of the 12 utilities, 11 completed the survey, but because of the anonymous nature of the responses, it was not possible to determine which utility did not respond or why. The survey consisted of 24 questions that could be answered as yes/no, short answer, or on a scale of 1–10. It was estimated that it would require about 10 min to complete the survey. A copy of the survey is found in Appendix S2.

2.6 | Data analysis

An Excel spreadsheet was provided to each participating utility so that a common set of data in a common format was collected. The spreadsheet also contained a look-up table so that the Legiolert MPN was automatically calculated once the number of positive wells was entered. The data from all the sites were compiled into a single file so that summary and descriptive statistics could be completed. The online survey data were downloaded to a spreadsheet file for analysis.

3 | RESULTS AND DISCUSSION

3.1 | *Legionella* and water quality

A total of 679 samples were analyzed during the study: 53 from the source water (Table 3), 50 from the plant effluent (Table 4), and 576 from the distribution system (Table 5). *L. pneumophila* was detected in three of five source water samples only at utility 7 and was not detected in untreated samples at any of the other utilities. One of three positive source water samples contained both serotype 1 and serotype 2–14, while the other two positive source water samples contained only serotype 2–14 strains of *L. pneumophila*. *L. pneumophila* serogroup 1 is the strain most commonly associated with waterborne disease outbreaks in the United States (Yu et al., 2002). The low frequency of detection is not surprising as the average source water temperatures ranged from 2 to 24°C, which is below the optimal growth threshold for *L. pneumophila* (Garrity, Bell, & Tilburn, 2005). Source water total coliform and heterotrophic plate count (HPC) levels showed good source water quality, with levels well below the criteria for source water used as a potable water supply (Bordner, Winter, & Scarpino, 1978).

L. pneumophila was not detected in any of the treated plant effluent samples (Table 4), in part because average free chlorine residuals ranged between 0.8 and 1.7 mg/L and total chlorine residuals ranged between 1.8 and 4.1 mg/L for the five utilities that practiced chloramination. In addition, all

TABLE 3 Summary of source water data

Utility code	*N*	Legiolert (mL avg)	Total coliform (mL avg)	HPC (mL avg)	HPC (mL min)	HPC (mL max)	Temp average (°C)	Temp min	Temp max	pH	pH min	pH max	TOC (mg/L)	TOC (mg/L min)	TOC (mg/L max)
1	4	0	ND	—	—	—	1.8	1.0	2.0	6.53	6.50	6.60	2.50	—	—
2	6	0	95	8	1	14	7.1	4.2	10.0	7.92	7.82	8.02	2.58	1.37	6.19
3	3	0	41	—	—	—	8.3	8.0	9.0	8.10	7.30	8.50	2.1	1.8	2.5
4	4	0	10	116	86	146	18.8	17.0	22.0	7.24	7.11	7.34	1.89	1.6	2.2
5	4	0	1,517	1,250	2	1,560	12.9	11.7	15.3	7.31	6.83	7.38	—	—	—
6	5	0	P	324	116	738	11.7	8.0	13.6	6.11	5.38	6.85	3.66	3.37	4.02
7	5	114	1,120	437	93	738	—	—	—	—	—	—	6.48	6.10	6.80
8	2	0	P	117	2	231	23.8	20.1	27.4	7.96	7.61	8.31	1.47	0.37	2.56
9	4	0	136	20	12	16	14.3	13.6	14.9	8.12	8.03	8.18	2.63	2.57	2.68
10	4	0	ND	—	—	—	4.3	3.4	5.0	7.00	6.90	7.10	—	—	—
11	5	0	A	4,200	—	—	6.2	—	—	7.41	7.36	7.55	2.30	—	—
12	7	0	475	132	51	299	3.9	1.4	6.6	8.10	8.00	8.20	7.90	6.50	10.60

Note. A: absent; Avg: average; HPC: heterotrophic plate count; Max: maximum; Min: minimum; ND: not determined; P: present; Temp: temperature; TOC: total organic carbon.

TABLE 4 Summary of plant effluent data

Utility code[a]	*N*	Legiolert (100 mL avg)	Total coliform (100 mL avg)	HPC (mL avg)	HPC (mL min)	HPC (mL max)	Total chlorine (mg/L)	Total min	Total max	Free chlorine (mg/L)	Free min	Free max	Temp (°C)	Temp min	Temp max	pH	pH min	pH max	TOC (mg/L)	TOC (mg/L min)	TOC (mg/L max)
1	4	0	0	0	0	0	2.1	2.0	2.2	—	—	—	4.0	4.0	4.0	8.40	8.30	8.50	2.50	2.50	2.50
2	9	0	0	0	0	0	—	—	—	0.8	0.6	1.1	7.0	2.1	12.8	7.29	7.11	7.42	—	—	—
3	9	0	0	0	0	0	2.4	2.2	2.8	—	—	—	7.2	5.0	9.0	7.19	7.04	7.38	1.53	1.10	1.99
5	2	0	0	0	0	0	1.2	1.1	1.3	0.9	0.7	1.0	16.4	15.9	16.8	6.73	6.70	6.76	—	—	—
6	2	0	0	0	0	0	—	—	—	1.1	1.0	1.1	16.4	16.2	16.6	7.70	7.60	7.80	1.65	1.53	1.77
7	4	0	0	1	0	2	4.1	3.8	4.4	—	—	—	21.0	20.4	21.7	8.03	7.95	8.11	4.05	4.00	4.10
8	2	0	0	0	0	0	—	—	—	1.1	1.0	1.1	27.1	26.4	27.7	7.68	7.67	7.69	0.50	0.49	0.50
9	8	0	0	0	0	0	2.0	1.9	2.2	1.7	1.5	1.8	13.6	12.9	14.1	7.82	7.61	7.99	—	—	—
10	1	0	0	0			1.8	—	—	—	—	—	5.0	—	—	8.90	—	—	—	—	—
11	2	0	0	1	1	1	—	—	—	0.8	0.8	0.8	8.6	7.8	9.4	7.75	7.74	7.76	1.30	1.30	1.30
12	7	0	0	0	0	2	3.8	3.5	4.0	—	—	—	5.7	2.6	7.8	9.02	8.93	9.11	3.56	3.05	4.34

Note. Avg: average; HPC: heterotrophic plate count; Max: maximum; Min: minimum; Temp: temperature; TOC: total organic carbon.
[a] Plant effluent data were not collected by Utility 4.

TABLE 5 Summary of distribution system data

Utility code	*N*	Legiolert (100 mL avg)	Total coliform (100 mL)	HPC (mL avg)	HPC (mL min)	HPC (mL max)	Total chlorine (mg/L)	Total min	Total max	Free chlorine (mg/L)	Free min	Free max	Temp (°C)	Temp min	Temp max	pH	pH min	pH max	TOC (mg/L)	TOC (mg/L min)	TOC (mg/L max)
1	50	0	0	4	0	74	1.4	0.3	2.0	—	—	—	6.5	4.0	10.0	7.79	7.30	8.00	2.50	2.50	2.50
2	36	0	0	0	0	0	—	—	—	0.6	0.1	1.0	7.7	2.1	15.0	7.30	6.38	7.81	ND	ND	ND
3	48	0	0	0	0	0	2.3	2.1	2.6	—	—	—	8.0	5.0	14.0	7.14	7.00	7.44	1.37	0.80	2.05
4	50	0	0	1	0	6	—	—	—	1.1	0.8	1.4	14.6	12.6	18.1	8.43	8.07	8.74	1.17	1.00	1.40
5	48	0	0	0	0	2	1.4	0.4	1.8	1.3	0.3	2.0	17.1	14.7	20.0	8.13	6.93	8.94	1.25	1.20	1.30
6	49	0	0	0	0	12	—	—	—	0.6	0.0	0.9	16.2	13.2	18.4	7.92	7.33	9.37	1.76	1.50	1.97
7	53	0	2	18	0	248	3.2	2.4	3.8	—	—	—	20.7	19.2	22.7	8.02	7.83	8.23	3.77	3.50	4.10
8	53	1	0	1	0	12	—	—	—	0.9	0.6	1.2	22.5	17.9	26.5	7.71	6.95	8.21	0.47	0.26	1.02
9	50	0	0	1	0	12	1.1	0.4	1.5	0.9	0.3	1.3	15.2	13.2	18.9	7.91	7.62	8.21	ND	ND	ND
10	50	0	0	1	0	38	1.4	0.4	1.6	—	—	—	9.2	7.2	12.7	9.02	8.40	9.60	1.93	1.93	1.98
11	47	0	0	17	0	450	—	—	—	0.4	0.0	0.8	7.9	6.1	9.6	8.31	7.56	9.72	1.18	1.10	1.30
12	42	0	0	0	0	3	3.4	2.6	4.5	—	—	—	6.8	1.4	14.9	9.03	8.82	9.17	3.39	2.99	4.02

Note. Avg: average; HPC: heterotrophic plate count; Max: maximum; Min: minimum; ND: not determined; Temp: temperature; TOC: total organic carbon.

total coliform samples were negative, and HPC levels were typically nondetectable. Generally, maintenance of a chlorine residual in potable water systems is effective for controlling *Legionella* spp. (Jjemba, Johnson, Bukhari, & LeChevallier, 2015; Kim, Anderson, Mueller, Gaines, & Kendall, 2002), but there are many situations where the bacteria can be shielded from the disinfectant (as in a biofilm or amoebae cyst), and therefore, complete eradication of the organism is difficult. Kuchta, States, McNamara, Wadowsky, and Yee (1983) reported a C × T (concentration times time) value of 0.5 min-mg/L at 21°C and pH 6.0 for 2-log (99%) reduction of *L. pneumophila* and values ranging from 1 to 6 mg-min/L and <3 to 9 mg-min/L for pH 7 and 7.6, respectively. In comparison, *Legionella* spp. in protozoa cysts survived 25-fold more chlorine disinfectant after 18 h (Kilvington & Price, 1990). Donlan et al. (2002) reported that monochloramine was significantly more effective than free chlorine at eradicating laboratory-grown biofilms of *L. pneumophila*. Lin, Stout, and Yu (2011) reported that, in a hospital in Washington, DC, a monochloramine concentration of 0.31 mg/L was effective in reducing *Legionella* counts in the building's plumbing system. Loret et al. (2005) found that planktonic *Legionella* decreased to undetectable levels after being dosed with 0.5 mg/L monochloramine in a model potable water pipe system for 3 days and remained undetectable for the remainder of the 1-month experiment.

A total of 576 distribution system samples were collected, including 26 (4.5%) from finished water reservoirs (Table 5). *L. pneumophila* was detected in only one sample (0.17%), whereas total coliform bacteria were detected twice (0.35%) in a distribution system unrelated to the positive *L. pneumophila* sample. *Escherichia coli* was never detected in any system during the study period. The positive *L. pneumophila* result at a concentration of 1 MPN/100 mL occurred in a sample that contained 0.72 mg/L free chlorine (pH 6.95, temperature 18.2°C, total organic carbon [TOC] of 0.32 mg/L) and was serotyped as belonging to the 2–14 serogroup. A repeat sample collected a week later was negative for *L. pneumophila.* Municipal water systems are not thought to be a major source of *Legionella* risk (Beer et al., 2015; Benedict et al., 2017). That said, low levels of *Legionella* may be able to break through treatment barriers entrapped in the cysts of free-living amoebae or inside protozoa hosts where they are protected from disinfection (Dupuy et al., 2011). Utility 8 is a groundwater system that is recharged by percolation of surface water through constructed infiltration basins where the recharged water mixes with the native groundwater. Wells then recover the blended water, which is treated and pumped into the distribution system. Riffard et al. (2001) detected *Legionella* by both cultural and molecular methods in both warm and cold groundwaters.

None of the 26 storage reservoirs examined in this study were positive for *L. pneumophila.* Elevated storage tanks may be prone to high water temperatures where water stratification may prevent mixing and subsequent loss of a disinfectant residual. Lu, Struewing, Yelton, and Ashbolt (2015) detected *Legionella* by quantitative polymerase chain reaction in 66.7% of municipal drinking water storage tank sediments from 18 sites. Diverse *Legionella* spp., including *L. pneumophila*, *L. pneumophila* sg1, and *L. anisa,* were identified. At least one outbreak has been associated with a community water system storage tank that had low (<0.2 mg/L) free chlorine residuals (Cohn et al., 2015).

None of the chloraminated distribution systems were positive for *L. pneumophila*, although two samples were total coliform positive (Table 5). These two samples contained 2.85 and 2.93 mg/L total chlorine, respectively, and repeat analyses of these sites were negative. Flannery et al. (2006) showed a 93% reduction in the occurrence of *Legionella* spp. in building plumbing systems in San Francisco after the utility converted from free chlorine to chloramines. Likewise, *Legionella* occurrence in Pinellas County, Florida, was reduced when the system converted from chlorine to monochloramine disinfection (Moore et al., 2006). Water samples were collected from 96 buildings (public buildings and individual homes) for a 4-month period when chlorine was the primary disinfectant and from the same sampling sites for a 4-month period after monochloramine was introduced into the municipal water system. When free chlorine was used, 19 buildings were colonized with *Legionella* in at least one sampling site. *Legionella* colonization was reduced by 69% (to six positive buildings) within a month after chloramination. Monochloramine appeared to be more effective in reducing *Legionella* in hotels and single-family homes than in county government buildings, perhaps because of more consistent water usage. In a 3-year study of monochloramine addition to a hospital in Italy, Marchesi et al. (2012, 2013) reported that a residual between 1.5 and 3.0 mg/L effectively controlled *Legionella* occurrence, with seven of the eight positive samples occurring within the first 8 months, and the eighth positive sample occurred at 15 months, when the monochloramine dose decreased below 1 mg/L. The authors suggested that a monochloramine concentration between 2 and 3 mg/L be maintained to assure *Legionella* concentrations less than 0.1 cfu/mL.

This study was not designed to assess the accuracy of the Legiolert method. Prior assessment of the test showed that the method had a specificity of 96.4% and yielded significantly higher counts of *L. pneumophila* than did the ISO 11731-2 membrane filtration (MF) method (Sartory et al., 2017). In that study, 14 of 290 samples (4.8%) yielded "false-positive" results—that is, the positive result in the Quanti-Tray did not serotype as an *L. pneumophila* strain. In this study, four (0.7%) distribution samples (three from utility 7, one from utility 6) failed to be confirmed by serotyping. The bacteria that grew in these samples were purified and sequenced for 16S by an outside laboratory (Genewiz, South Plainfield, NJ) and identified as *Brevundimonas vesicularis,*

Sphingomonas koreensis, Ochrobactrum intermedium, Brevundimonas dimunata, and *Elizabethkingia anophelis*. It is recommended that utilities serotype all positive Legiolert samples—both to confirm that *L. pneumophila* is present and to better understand the public health significance of the isolates. According to the CDC, most disease is caused by *L. pneumophila*, particularly serogroup 1 (CDC, 2017).

3.2 | Post-study survey

The scope of this study was not intended to be reflective of all U.S. water utilities, nor was the timing of this study optimal to capture the summer season when water temperatures, and the opportunity for bacterial growth, would be the highest. Plans are underway to conduct additional summertime monitoring. The main objectives of this study were to assess utilities' experiences with using the Legiolert test and to develop a protocol for responding to positive samples. A post-study survey was conducted to evaluate the utilities' motivation and experiences in conducting Legiolert monitoring.

Monitoring for *Legionella* in potable water systems is not required by any state or federal regulations, and as already mentioned, many of the utilities initially contacted were concerned about finding *Legionella* in their water systems. However, the motivation for the utilities that did participate in the study was primarily to better understand *L. pneumophila* occurrence in their system, as well as to become familiar with the Legiolert method and to "get ahead of any future regulations" (Figure 2).

There were concerns voiced during the participants' decision process to join the study. About two-thirds of the participating utilities were concerned with how to respond to positive *L. pneumophila* samples and/or how to communicate the information to state regulators and/or the public (Figure 3). When the participants did inform their regulators of the study, several were supportive; others were cautious or uncertain how their agency would respond to positive *L. pneumophila* results. Even though the laboratories agreed the method was simple to use, there was still a concern regarding the workload of processing nearly 700 additional samples and learning a new methodology.

Figure 4 summarizes the utilities' responses to a series of questions on the ease of use of the Legiolert test. All the responses scored an 8 or better on a scale of 1–10, with 10 being the highest rating. Generally, the analyst found the test easy to use, reported that the results were clear to read, and felt confident in using the test to detect *L. pneumophila* in source and treated waters. One of the analysts said, "The nice part about this method is that it is very straightforward …." Others said, "Training was straightforward" and "Instructions and protocol were easy to follow." Learning a new test and adding more than 60 additional samples (including QA/QC) to an already busy laboratory workload resulted in the question about "analyst's workflow" scoring the lowest response (slightlybelow 8 out of 10), but one supervisor clarified, "It took some time, but it was worth the effort." The main concern about the test was the need for an extra incubator and the fact that the source water and potable water tests have slightly different protocols, including pretreatment steps and incubation temperatures. One lab manager reported, "I was a little concerned the lab techs would remember to switch techniques for the potable vs non-potable assay, but none of the labs reported any problems keeping the procedures straight."

When the participants were asked about the need for monitoring of distribution systems for *L. pneumophila*, about two-thirds of them supported routine testing (Figure 5), citing, for example, that *"L. pneumophila* is the leading cause of water related outbreaks." However, 27% of respondents were undecided, stating, for instance, that they would "wait until research or regulatory agencies provided guidance on the appropriate application of such a test or other tests." Nonetheless, one respondent stated, "It would seem that a well-run distribution system with active programs to maintain and improve disinfectant residuals (and are successfully doing so) wouldn't benefit from routine testing for *Legionella*." All three viewpoints are understandable. *L. pneumophila* is the most commonly identified pathogen associated with drinking water outbreaks (Beer et al., 2015), and risks from a well-operated and disinfected distribution system are thought to be small. At the same time, the lack of U.S. guidelines for monitoring and responding to *Legionella* occurrences in distribution systems has been a deterrent to utilities conducting more monitoring.

3.3 | Protocols for responding to *L. pneumophila* occurrences

In the development of this project, the project team worked with regulators from the State of Washington to craft

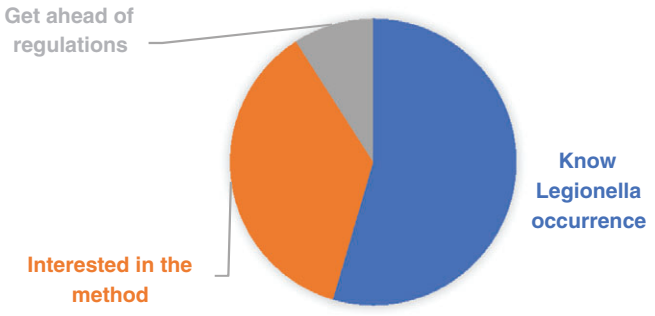

FIGURE 2 Motivation for participating in the study

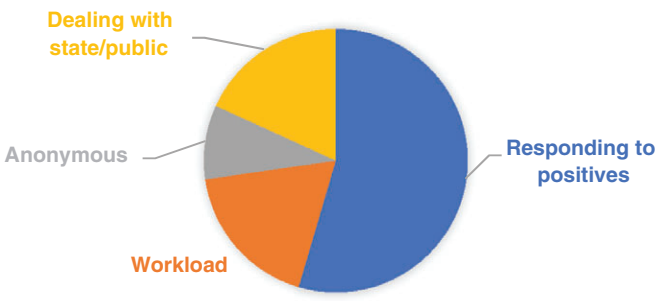

FIGURE 3 Concerns voiced during the decision-making process

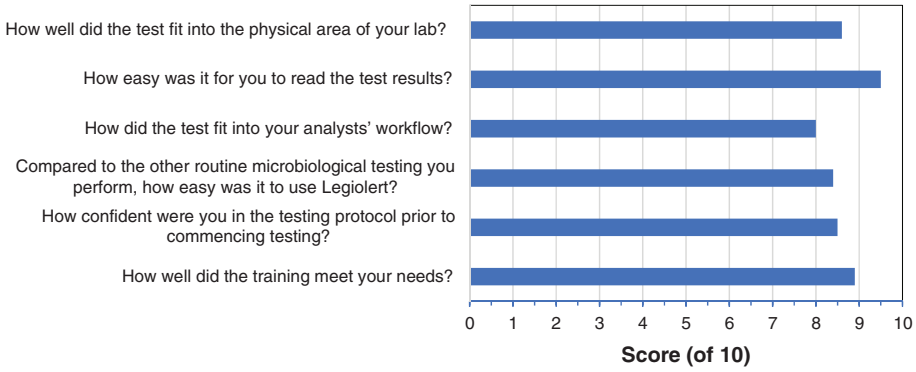

FIGURE 4 Utility survey responses on the ease of use for the Legiolert test

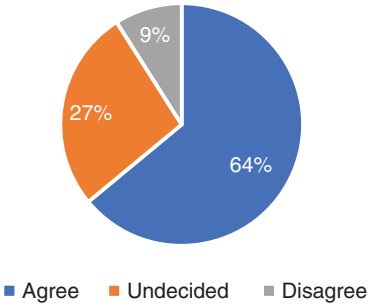

FIGURE 5 Support for distribution system monitoring for *Legionella pneumophila*

protocols similar to those shown in Table 6 for responding to positive distribution system *L. pneumophila* samples. These suggestions were shared with participating utilities as optional reference documents. All positive samples should be serotyped or speciated to demonstrate that *L. pneumophila* is present. In this study, three positive Legiolert samples failed to be confirmed by serotyping. Repeat testing of the one positive *L. pneumophila* (belonging to the 2–14 serogroup) sample was negative, and the original sample contained 0.72 mg/L free chlorine (pH 6.95, temperature 18.2°C, TOC of 0.32 mg/L). In this case, the random occurrence of *L. pneumophila* was not an indication of any failure of the water treatment processes.

Although the situation was not encountered in this study, a "Level 1 TCR" assessment was recommended if multiple samples were positive for *L. pneumophila* from the same site (Table 6). The Revised TCR (RTCR; USEPA, 2013) requires a Level 1 assessment to be conducted by the public water system owner and assesses any mechanism that could provide a pathway for microbial contamination or any sanitary defect that indicated a failure of a protective barrier to prevent microbial contamination. In the context of *Legionella* contamination, such an assessment could include any failures in treatment or disinfection, main breaks, failures in storage tanks or reservoirs, cross connections, backflow, and so on. Similar to the RTCR, any defects identified would be expected to be corrected. Such actions could include flushing parts of the network with low disinfectant residuals, cleaning of storage tanks, or boosting disinfectant levels within the distribution system.

Neither the USEPA nor the CDC has set specific trigger levels for acceptable concentrations of *L. pneumophila* in potable water supplies. The Occupational Safety and Health Administration (OSHA) does not have specific standards for Legionnaires' disease but had suggested action level guidelines

TABLE 6 Protocols and general guidelines for responding to *Legionella* occurrences in potable water systems

Positive sample procedures	Positive samples should be speciated/serotyped. A repeat sample should be collected from the sample site within 24 h of reporting the positive sample. If the repeat sample is negative, the site should be resampled at regular intervals as per study protocols. A second positive *Legionella pneumophila* sample should trigger a Level 1 TCR assessment to see if there are any recent events in the system that could have accounted for the positive result. Check in with DOH/regulator. Flush the area near the positive site, particularly if disinfectant levels are low. If the positive is from a reservoir, consider draining and cleaning the reservoir.
General guidelines	Compare water quality data (free/total chlorine, etc.) for the site compared with historical levels. Determine if any anomalies exist. Trigger levels can be established to prompt corrective actions. • OSHA guideline concentrations or European Union guidance can be considered in establishing possible action triggers. • Triggers could be set based on the frequency of occurrence (e.g., 5–20%). Multiple detections/high densities of *L. pneumophila* in the distribution system should trigger close consultation between the utility and regulator regarding follow-up actions and communication to other parties.

Note. DOH: department of health; OSHA: Occupational Safety and Health Administration; TCR: Total Coliform Rule.

TABLE 7 Action levels[a] for *Legionella* in water (in cfu/mL)

Action	Cooling tower	Domestic water	Humidifier
1	100	10	1
2	1,000	100	10

Note. Occupational Safety and Health Administration policy no longer supports these guidelines but reflect historical recommendations (OSHA, n.d.).
[a] Action 1: Prompt cleaning and/or biocide treatment of the system. Action 2: Immediate cleaning and/or biocide treatment. Take prompt steps to prevent employee exposure.

TABLE 8 Action levels for *Legionella* spp. in potable hot and cold water systems

Legionella (cfu/mL)	Action required
Not detected	None
<0.1 to 1.0	Assure water quality values are within target
1.0–10	• Resample if small percentage (10–20%) are positive; review control measures • If >20% positive, disinfection of system, risk assessment
>10	Resample, conduct immediate review of control measures, perform disinfection of whole system

Source: Adapted from EU (2017).

(Table 7) to assess the effectiveness for water system maintenance (OSHA, n.d.).

International guidelines, however, mirror the prior OSHA recommendations for building water systems maintenance. The European Union (EU) published guidelines for the prevention, control, and investigation of infections caused by *Legionella* species (EU, 2017). The guidelines emphasize proper building water management plans focusing on obtaining proper temperature, biocide, operations, and maintenance programs. Monitoring to demonstrate the effectiveness of the plans should meet the values shown in Table 8. The guidelines emphasize the goal to achieve no culturable *Legionella* but acknowledge that occasional detection (<20%) of low levels of *Legionella* (<1 cfu/mL) may be acceptable provided that other water quality values (e.g., temperature, disinfectant) and operational parameters were within the water management plan guidelines. Intermediate (1–10 cfu/mL) and high (>10 cfu/mL) occurrence would trigger a series of actions, including resampling, disinfection, and overall review of the water management plan program. Triggering remedial actions results in prompt responses to protect public health while still providing the flexibility for water systems to deal with low, sporadic detections of *Legionella* in water.

The lack of clear guidelines for potable water systems from the USEPA or the CDC have hampered the collection of data on *Legionella* occurrence by building owners and water utilities over fears that a single detection could trigger onerous remediation requirements by public health and environmental regulators. In fact, monitoring for *Legionella* in the absence of any problems has not been recommended (CDC, 2016) and the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) 188 standard does not require monitoring as part of the building water management plan (ASHRAE, 2015). Development of recommendations similar to Table 8 for potable water systems would provide a framework for responding to positive *Legionella* samples while allowing for flexibility for dealing with *Legionella* occurrences.

It is important to note that these guidelines are not intended as enforceable regulations, nor are they risk-based, but they do reflect practical operational experience. In this study, the concentration of *L. pneumophila* (0.01 MPN/mL) did not approach the thresholds outlined in the OSHA or EU guidelines (EU, 2017). However, the response protocols would have been useful if there had been a need to engage the state regulatory agencies or other stakeholders. Additional research will be needed to establish specific trigger levels for drinking water distribution systems. These trigger levels will be informed by the baseline monitoring for well-operated potable water systems. The development of a framework for responding to *Legionella*-positive drinking water samples will be useful until such a time when health-based standards can be developed.

4 | SUMMARY AND CONCLUSIONS

Most outbreaks of Legionnaires' disease are attributed to cooling towers or the plumbing in large buildings such as hospitals or hotels (Beer et al., 2015; Benedict et al., 2017). The drinking water distribution is not thought to be an environment where substantial proliferation of *Legionella* occurs. Still, the water utilities and the building managers have a shared responsibility to manage both networks (the distribution system and the building plumbing) to limit the occurrence and concentration of *Legionella* in water. The objective of this study was primarily to assess the utilities' experiences with using the Legiolert test. In addition, the project developed a protocol for responding to positive samples. The experience gained in this study will help utilities be proactive in managing *Legionella* occurrences in their systems. Overall, the participants found the Legiolert assay easy to use and interpret. The need for an extra incubator and the differences between potable and nonpotable Legiolert protocols were the only major concerns in using the test. Most importantly, the participants felt confident and well trained in using the test. In total, 679 water samples were processed by 12 utilities. *L. pneumophila* was detected in 5.7% of 53 source water samples; none of the 50 plant effluent samples; and in 0.17% of 576 distribution system samples, including those collected from reservoirs and storage tanks. Serotyping or speciation is recommended for all Legiolert-positive samples—both to confirm that *L. pneumophila* is present and to better understand the public health significance. This study was conducted during cold weather months, and a second study is underway during warm summer temperatures. Many additional utilities were interested but reluctant to participate in the study because of

the lack of guidelines on how to respond to positive *L. pneumophila* samples. In collaboration with a state agency, the project team developed protocols consistent with OSHA and EU guidance for responding to positive samples. Most of the utility participants said their main motivation for participating in the study was to understand *L. pneumophila* occurrence in their distribution systems so that they could improve treatment if any problems were found. This approach is the basis for protecting public health—to be constantly learning and continuously improving. The benefit of this study was not because *L. pneumophila* was infrequently detected in drinking water supplies but because a dozen utilities improved their understanding of how to produce safe drinking water.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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