Widespread Molecular Detection of *Legionella pneumophila* Serogroup 1 in Cold Water Taps across the United States

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**ABSTRACT:** In the United States, 6,868 cases of legionellosis were reported to the Center for Disease Control and Prevention in 2009–2010. Of these reports, it is estimated that 84% are caused by the microorganism *Legionella pneumophila* Serogroup (Sg) 1. *Legionella* spp. have been isolated and recovered from a variety of natural freshwater environments. Human exposure to *L. pneumophila* Sg1 may occur from aerosolization and subsequent inhalation of household and facility water. In this study, two primer/probe sets (one able to detect *L. pneumophila* and the other *L. pneumophila* Sg1) were determined to be highly sensitive and selective for their respective targets. Over 272 water samples, collected in 2009 and 2010 from 68 public and private water taps across the United States, were analyzed using the two qPCR assays to evaluate the incidence of *L. pneumophila* Sg1. Nearly half of the taps showed the presence of *L. pneumophila* Sg1 in one sampling event, and 16% of taps were positive in more than one sampling event. This study is the first United States survey to document the occurrence and colonization of *L. pneumophila* Sg1 in cold water delivered from point of use taps.

**INTRODUCTION**

*Legionella pneumophila* is an environmental microorganism capable of causing a range of adverse health effects from severe Legionnaires’ disease to moderate Pontiac fever influenza-like symptoms in humans. In 2009–2010, CDC’s National Notifiable Disease Surveillance System (NNDSS) reported 6,868 cases of legionellosis.1,2 Only 2.6% (179/6,868) of these cases were outbreak events associated with drinking water, other nonrecreational waters, or recreational waters.3,4 The remaining 97.4% cases of legionellosis were characterized as sporadic (e.g., not associated with an outbreak).

According to the CDC’s outbreak reports, exposure to *Legionella* is through potable water when other nonrecreational uses such as ornamental fountains and cooling towers are included3,4 with drinking water. Independent of exposure route, legionellosis is primarily acquired from aerosolized water droplets contaminated with *Legionella* microorganisms.

Water contamination by *Legionella* is currently monitored by many countries utilizing conventional culture methods based on International Organization for Standardization (ISO) 11731, The Netherlands Normalizatie-Institute (NEN) 6265, The Association Francaise de Normalization (AFNOR) NF T90-431.7,8 methods. However, as is the case with all methods, there are limitations when culturing *Legionella*. Optimal cultivation requires amino acid supplementation, attention to pH sensitivity, and prolonged incubation periods (up to ten days). With a generation time of 4 to 6 h, overgrowth of cultures with other microorganisms is likely to occur.9,10 Another factor limiting the culture-based detection of *Legionella* is the physiological state of the cells. For instance, viable but nonculturable (VBNC) *Legionella* cells and cells present within amoeba will not be detected by conventional culture methods.11,12

The use of qPCR for the purpose of surveillance has some merit because qPCR detects and amplifies a specific gene target known to be exclusive to a specific genus/species/serogroup. The qPCR technique, with the use of a standard curve, is far more effective and efficient at quantifying the presence of a specific microorganism than the traditional culture approach. To date, several qPCR assays for *Legionella* have been developed: a genus-specific assay (targeting the 16S rRNA gene from *Legionella* spp.),13 a species-specific assay (targeting the *mip* gene, coding the macrophage infectivity-potentiator of...
**MATERIALS AND METHODS**

**Sampling Sites.** Forty geographically dispersed sites (32 buildings and 8 houses) in 25 states, 1 territory, and 1 federal district within the US were monitored for the occurrence of *L. pneumophila* Sg1, from January 2009 to December 2010 (Figure 1). At each site, one (*n* = 12) or two (*n* = 28) cold water taps were selected for monitoring. In total 68 taps - 29 kitchen sinks, 21 bathroom sinks, 17 drinking water fountains, and 1 refrigerator door water dispenser were monitored. The water analyzed was used as the home or building potable water source. No site had a secondary water treatment device installed, with the exception of the refrigerator door which had an in-line filter. The monitoring of two taps per site allowed U.S. EPA to evaluate the extent of the exposure to *L. pneumophila* Sg1 within a site.

![Geographical map of United States - location and site type in study](image)

**Figure 1.** Geographical map of United States - location and site type in study. △ Indicates site as a house and ■ indicates site as a building. The number insert indicates the number of taps at the site.

In 2009, water was collected from each tap three times during the year. In 2010, water was collected once from the same sites. At all taps, the water was collected in three, one liter high density polypropylene (HDPP) bottles, fifteen seconds after the water started flowing; sodium thiosulfate was not present in the sample collection bottle. The fifteen second flush was performed to ensure that the water collected came from the cold line behind the hot and cold interface. The water was shipped next day delivery with ice packs, and samples were vacuum filtered within 48 h of its collection. Water quality reports from the water distribution authority were obtained for the locations (*n* = 38) represented by the samples.

**Samples and DNA Extraction Used for qPCR Analysis.**

Two hundred and seventy-two DNA extracts were generated from the collected samples. The extracts were the result of vacuum filtering 3 L of water through a sterilized Nucleopore Track-Etch, 47 mm, 0.4 μm polycarbonate membrane (Whatman Inc., Piscataway, NJ). The filters were stored at −80 °C in sterile 2.0 mL O-ring screw cap microcentrifuge tubes containing 0.30 ± 0.05 g 0.1 mm of glass beads (BioSpec Products, Bartlesville, OK) until extraction.

The DNA was extracted from each sample using a modified WaterMaster DNA extraction protocol (Epigen Biotechnologies, Philadelphia, PA). Details of this extraction have been published previously. In brief, each polycarbonate membrane was bead-beaten in a bead-beater (BioSpec Products, Bartlesville, OK) for 3 min on the “homogeneity” setting with 0.05 g of Tissue and Cell Lysis Solution (Epigen Biotechnologies, Philadelphia, PA). The lysate was transferred to 2 mL microcentrifuge tubes, and 2 μL of Proteinase K (50 μg/μL) (Epigen Biotechnologies, Philadelphia, PA) was added and incubated at 65 °C in a water bath for 15 min. Next, 2 μL of RNase A (5 μg/μL) (Epigen Biotechnologies, Philadelphia, PA) was added followed by incubation in a 37 °C water bath for 30 min. Samples were placed on ice for 5 min, and 350 μL of MPC Protein Precipitation Reagent (Epigen Biotechnologies, Philadelphia, PA) was added. The supernatant was transferred to a sterile microcentrifuge tube, and an equal volume of ice cold (∼−4 °C) isopropl alcohol was added. The samples were inverted manually up to 40 times and then centrifuged at 10,000g for 10 min. The isopropl alcohol was poured off, and the resulting DNA pellet was washed with 500 μL of ice cold (∼−4 °C) 70% ethanol. Samples were centrifuged, and the ethanol was removed. The pellets were resuspended in 150 μL of nuclease-free sterile water and stored at −80 °C until analyzed.

**Bacterial Culture and Preparation of Standard.** *L. pneumophila* Sg1 ATCC 33152 (ATCC, Manassas, VA) and other strains (Table S1) were cultivated on Buffered Charcoal Yeast Extract (BCYE) agar base containing 10% l-cysteine (BD, Franklin Lakes, NJ) at 35 °C under microaerobic conditions (Mitsubishi Gas Chemical America, New York, NY). After 48 h, a 10 μL loopful of cells was harvested from BCYE agar plates, and the DNA was extracted as described above. The extracted DNA was quantified on a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE). For the specificity tests, DNA was diluted to 1 ng/μL and stored at −80 °C until analyzed by both assays.

A standard curve was prepared by using *L. pneumophila* Sg1 ATCC 33152 by calculating 10^x target gene copies per qPCR reaction from the 4.3 fg genomic mass of *L. pneumophila*. Next, a (1:10) serial dilution was performed down to a “theoretical” one target gene copy per qPCR reaction. In each...
25 μL qPCR reaction, 5 μL of standard template was analyzed per qPCR reaction equating to 100 mL of the original volume.


Quantitative PCR (qPCR) Conditions. Two primer probes sets were used to detect and quantify L. pneumophila and L. pneumophila Sg1 in water. All DNA extracts were analyzed using the Lp16S primer-probes set, which is specific for L. pneumophila. If the extract was positive for this target, the second primer-probe set LpLPS, which is specific for L. pneumophila serogroup 1 (Sg1), was used to confirm the L. pneumophila detection as well as provide Sg-specific information.

The qPCR reaction targeting the 16S rRNA gene contained the following: 17 μL of TaqMan Universal PCR Master Mix, 2.0 μL of a mixture of forward and reverse primers (500 nM) and a 100 nM IDT Probe, 1 μL of 0.1% Bovine Serum Albumin (BSA), and 5 μL of DNA purified.

The Lp16S primer-probes set targeted a 100 basepair (bp) sequence of the 16S rRNA gene was developed using TaqMan chemistry, in-house.30 The probe and primers (IDT, Corelville, IA) for the Lp16S assay were the following:
• probe Lpneu P1: 5′-6FAM-AAGCCCAAGGAATTCACAGAT-TAMRA
• forward primer, Lpneu F1: 5′-CGGAATTACTGGGCG-TAAAAG-3′
• reverse primer, Lpneu R1: 5′-GAGTCAACAGTAT-TATCTGACC-3′

Reactions were performed in triplicate using a Roche LightCycler 480 II Real-time PCR system (Roche Applied Science, Indianapolis, IN). The LightCycler setting included monocholor-hydrolisys detection with a preincubation step of 10 min at 95 °C, an amplification step of 45 cycles of 10 s at 95 °C, 30 s at 60 °C, 1 s at 72 °C, and a cooling step of 30 s at 40 °C.

A sample was considered positive for L. pneumophila if two or more of the replicates had a quantification cycle (Cq) value <40. If this criterion was met, positive samples were analyzed with the LpLPS assay to confirm the presence of L. pneumophila. The L. pneumophila serogroup-specific probe and primer sequences targeted a 75 bp sequence of the LPS cluster gene. The probe and primers (IDT, Corelville, IA) for the LpLPS assay were the following:
• probe SG1-pb: 5′-6FAM-TCTTGAGATGGTTGGAT-TTA-BHQ
• forward primer P65 F1: 5′-CAAAGGGCGTTACAGTCAAACC-3′
• reverse primer P66 R1: 5′-CACAACCCCAAACCGTATTCA-3′

Reactions were performed in duplicate, and both replicates were required to have a Cq value <40 to be considered positive.

Controls. A number of controls were included on each plate to ensure the integrity of the method and confidence in the results. Genomic DNA extracted from L. pneumophila Sg1 ATCC 33152 was used as a positive control. A serial dilution of seven concentrations ranging from 10^6 to a theoretical one genomic copy was made from this DNA. Negative control measures included three no template controls (NTC), where sterile water was used in place of the DNA extract (template).

Method blanks were established at the time of the water filtration. One hundred milliters of sterile molecular grade water (5 Prime, Gaithersburg, MD) were vacuum filtered as described above, for every 10 samples filtered. If a method blank was positive, the set of samples that corresponded with it was considered compromised, and the data discarded. QPCR inhibition was monitored using an external control. In a separate qPCR plate, all unknown samples were spiked with 1 μL of an exogenous control of 10,000 target gene copies extracted from L. pneumophila ATCC 33152. A reaction was considered inhibited it the observed Cq value drifted ≥1.5 Cq units from the standard Cq value.

Limit of Detection and Limit of Quantification for Standards. The limit of detection (LD) and the limit of quantification (LQ) for standards of the Lp16S and LpLPS assays were determined using serial dilutions of genomic DNA obtained from ATCC 33152. The LD for standards was defined as the lowest copy number giving a Cq < 40 and was determined from seven independent dilution series. The LQ for standards was defined as the lowest copy number per assay yielding a coefficient of variance less than 25%.14

Limit of Detection and Limit of Quantification for Sample Process. A study was conducted in a series of spiked samples to determine LD and LQ for the process. The selected water samples represented four common potable water types; chlorinated surface water (three sites), chlorinated groundwater (one site), chloraminated surface water (one site), and untreated groundwater (one site). For each water type, five 1 L samples were spiked with five known quantities of Legionella cells (an averaging of direct cell counts and plate counts) spanning 1 CFU - 10^6 CFUs. Each liter was vacuum filtered through Nucleopore Track-Etch, 47 mm, and 0.4 μm polycarbonate membrane. The DNA extraction and both qPCR assays were performed as previously described. The LD of the process was defined as the lowest CFU that had a Cq < 40, 100% of the time.

Statistical Analysis. The Cq values were initially transformed to genomic target numbers using the standard curve generated from the serial dilutions of L. pneumophila Sg1 genomic DNA. The averaging of the genomic target number per replicate was calculated for each sample that had a Cq < 40. For taps that had a positive detection, a median value was calculated. Results were exported as a tab-delimited data file. The data were read into the software program R (version 2.12.1) (www.r-project.org), followed by log-transformation prior to tests based on normal approximations to reduce heteroskedasticity and skew. The Kolmogorov–Smirnov test (stats), Kruskal-Wallace test, Wilcox test, Student’s t test, and Fisher’s exact test were conducted using functions in the ‘Stats’ package, while resampling was conducted using functions in the base package.

RESULTS AND DISCUSSION

Specificity and Sensitivity of qPCR Assays and Method. Specificity of the two primer/probes sets (Lp16S and LpLPS) were tested against the DNA extracted from 37 strains: 19 strains of L. pneumophila, 12 strains of other Legionella species, and 6 strains of other bacterial genera (Table S1). Lp16S assay had a Cq signal of <40 for all of the L. pneumophila strains tested and had no cross reactivating with the other Legionella spp. and other genera. The LpLSP assay was only reactive with those strains known to be L. pneumophila Sg1.
The performance of each qPCR assay was evaluated against three measures: regression analysis (linearity), amplification efficiency \( (E = 10^{(-1/\text{slope})}) \), and consistency across replicate reactions as measured by relative standard deviation (RSD). The standard curves for both assays were highly linear having \( R^2 \) of 0.999 for the Lp16S assay and 0.995 for LpLPS assay. The amplification efficiency for both assays was also very similar with values of 95.8% for the Lp16S assays and 96.2% for the LpLPS assay. The relative standard deviation (RSD) at each assay’s LD did not vary greatly between replicates performed on a daily basis or over a six week span.

The LD and the LQ for the Lp16S assay was determined to be one “theoretical” genomic target/reaction, for both measures. The LD and LQ for the LpLPS assay were 10 genomic targets/reaction for both measures.

Regardless of water quality type, \( L. \) pneumophila spiked at 100 cells/L was detected 83% of the time and a spiked of 1,000 cells/L was detected 100%, for both assays. These recovery rates are well below the 1,000 to 10,000 CFU/L necessary for detection by culture.9

Occurrence and Colonization of \( L. \) pneumophila Sg1 in Tap Water by qPCR. Two hundred and seventy-two samples drawn from a cold tap water test were examined for the presence of \( L. \) pneumophila Sg1. Of the original 272 samples, nine samples (3.3%) completely inhibited the qPCR reaction for the Lp16S assay. These nine samples were subsequently analyzed by the LpLPS assay; where upon six samples of the nine observed no inhibition and were classified as negative. Three samples remained inhibited and removed from further analysis. Of the remaining 269 samples, 77 (28.6%) were positive for \( L. \) pneumophila with the first Lp16S assay.

In this study, a “positive sample” was defined by the detection of both gene products in a sample having a \( C_q < 40 \). Only samples that met these criteria are reported here as positive. Twenty percent (53/269) of the water samples were found to be positive for both assays. Two hundred sixteen samples (80%) were negative (Table 1). Using two targets to determine if a sample was positive for \( L. \) pneumophila Sg1 ensures that the DNA had come from an intact cell. This added measure helps reduce the number of false positives but is not a failsafe, because any recently dead cells with an intact cell membrane can still test positive.

The samples represent water that was collected from 68 taps over the course of two years. Fifty-three percent of the taps (36/68) were consistently negative for \( L. \) pneumophila Sg1. The remaining 32 taps (47%) were positive for \( L. \) pneumophila Sg1 at least once (Table 1). Five taps (7%) were positive for \( L. \) pneumophila Sg1 at all four sampling events. Two taps (3%) had positive detection in three of the four sampling events, and four taps (6%) had positive detection in two of the four sampling events. \( L. \) pneumophila Sg1 was only detected once for the remaining 21 taps (31%) (Figure 2).

The samples for the taps that had positive detections were collected over a period of 11–20 months. Thus, a person could be exposed to this microorganism at various concentrations, more than once from their household- or facility-water during that time period. Additionally, from among those sites that showed more than one occurrence of \( L. \) pneumophila Sg1, both taps within the site were consistently positive for the microorganism in all cases. Flannery et al. (2006) in their study examining the effectiveness of monochloramine in reducing Legionella colonization of water systems also observed that samples from many taps or sites were consistently positive for Legionella spp. over a period of 12 months.31 Although we found no increased risk of exposure associated with the “kind” of tap, one of the highest concentrations of \( L. \) pneumophila Sg1 detected came from a facility drinking water fountain [LPS assay \( \mu = 2.18 \times 10^5 \pm \text{SE} 1.3 \times 10^4 \) genomic target/L]. Overall, 47% of facility drinking water fountain samples collected during our study were positive for \( L. \) pneumophila Sg1 at least once and about 18% (3/17) consistently tested positive.

Published reports that use qPCR for detecting and monitoring for \( L. \) pneumophila in potable water have mostly originated from Europe. This study’s frequency of detection at the sample level (27%) was lower for qPCR than reported in other countries (Spain 52%,23 France 73%,14 47%,32 Belgium 47%).33 The only other study that analyzed potable water for \( L. \) pneumophila Sg1 determined that 19% of their cold water samples were molecularly positive, a lower value than the 27% reported in this study.36

There have been very few studies conducted in the U.S. that examined the occurrence/colonization of Legionella at point of use taps, other than at health institutions. Stout et al. (1992) examined the homes of members of the American Legion in Pittsburgh and found 6.4% (14/218 tap) positive for Legionella spp.34,35 In 2006, two studies were published that examined the colonization of Legionella within two separate public water service areas,31,36 The colonization of Legionella spp. at the point of collection was 7.8% (15/192) in one study and 38.7% (246/636) in the second. Each of these studies used culture methods to determine occurrence/colonization and limited their sample collections to a specific city or region. Therefore, it is difficult to make comparisons to our results.

<table>
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<th>Table 1. Results of Lp16S and LpLPS Assays</th>
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<tr>
<td>Lp16S/LpLPS (+/+ positive)</td>
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<tr>
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<tr>
<td>samples</td>
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<td>53 (20%)</td>
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<td>32 (47%)</td>
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<td>68 (20%)</td>
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![Figure 2](https://example.com/figure2.png)
where the samples were collected from geographically disperse areas.

Quantification of *L. pneumophila* Sg1 Occurrence in Cold Water Tap Samples. qPCR, combined with disruptive cell lysis, is a molecular enumeration technique best suited for establishing maximum potential exposure levels for a sample because it quantifies live cells, those encysted in amoeba, plus intact dead cells. These other states of *Legionella* would be otherwise unmeasurable if culture was the sole enumeration method. Moreover, qPCR also supplies quantitative values that can be used for risk assessment given consideration of the appropriate assumptions and risk factors.

Among the 53 water samples that were positive for *L. pneumophila* Sg1, 50% had concentrations that ranged from 40 to 620 genomic targets (LPS)/L. The mean and median were 1.97 × 10^5 genomic targets (LPS)/L and 62 genomic targets (LPS)/L, respectively. The minimum and maximum concentrations were 40 genomic targets (LPS)/L and 3.65 × 10^6 genomic targets (LPS)/L. The sample that had the maximum concentration was not from a public drinking water system.

Figure 3A shows the distribution of the calculated median *L. pneumophila* concentrations measured in the positive samples for each tap evaluated using the *L. pneumophila* (Lp16S) assay and the *L. pneumophila* Sg1 (LpLPS) assay. Of the 32 positive
taps, 66% had concentrations (LPS assay) below culture LD. This indicates that for that vast majority of the positive samples, the concentrations of *L. pneumophila* Sg1 were very low. The concentrations of colonized taps are presented in Figure 3B. Among the eleven taps that were considered colonized, 27% (3/11) had concentration of >1 × 10^3 genomic targets/L.

European countries such as France, The Netherlands, and The United Kingdom have established >1,000 CFU/L as a guideline for corrective action when *Legionella* is found in public water systems. The US’s Department of Labor’s Occupational Safety and Health Administration’s (OSHA) technical handbook for industrial hygienists has two action levels for domestic water. If levels are detected above 10,000 CFU/L (Action Level 1), both U.S. and Europe recommend repeat sampling and promptly cleaning or adding a biocide to the system. If concentrations climb to 100,000 CFU/L (Action Level 2) immediate cleaning, biocide treatment, and prompt steps to prevent employee exposure must be done. The American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE) recently proposed a new standard for prevention of legionellosis in building water systems for their industry (Standard P188). ASHRAE’s proposed standard is only applicable to occupied buildings over 10 stories high that share a centralized heated water system and/or inpatient healthcare facility that may have occupants older than 65 years and contain two or more water features (e.g., water fountains) and/or whirlpools/spas. This proposed standard (P188) would require the water in such a building to have a free chlorine residual of 0.5 ppm.

As validated molecular methods for identifying microbial contamination become more common, there is a need to determine the relationship of the molecular measurement units to the standard culture of CFU values. In recent years, an effort has been made by researchers from six European countries to derive both alert and action qPCR thresholds for *Legionella* and *L. pneumophila* in cooling towers and hot and cold water systems. These researchers developed a decision-based flowchart for the interpretation of qPCR results from the buildings evaluated. Select locations were monitored over a course of ten weeks employing both culture and two commercial qPCR assays, which targeted *Legionella* spp. and *L. pneumophila* in order to generate the data set that was used to recommend qPCR alert and action levels for *Legionella*. An alert level of >5 × 10^3 Genomic Unit (GU)/L and >4 × 10^3 GU/L of *L. pneumophila* was recommended for cooling towers and hot and cold water systems, respectively. By applying this suggested alert level to the Lp16S data set in this study, only four taps exceed the proposed alert limits.

**Disinfectants Used in Potable Water Treatment.** Disinfectants such as chlorine and monochloramine are used to reduce the indigenous microbes in source water. In recent years, studies have shown *Legionella* colonization within a water system is significantly reduced when a municipality converts to the use of monochloramine as its disinfectant. In a retrospective cohort study of 15 hospitals, there was a strong statistical association between disinfection with monochloramine and absence of *Legionella* in the water. Monochloraminated water was also found to produce a statistically significant reduction in the risk for hospital-acquired legionellosis.

Of the 68 taps sampled in this study, 66 taps received treated water from a public utility. Based on the retrieved water quality reports, 43 taps (63%) received chlorinated water and 23 taps...
(37%) received water treated with monochloramine. Frequency of detection of *L. pneumophila* Sg1 in a sample was similar for both disinfectant types. However, taps classified as colonized based on more than one detection were observed more frequently from systems that used chlorine (7/43, 16%) than systems that used chloramine (2/23, 8.6%).

Statistical analysis of concentration (genomic target/L) data did not find a significant difference between the chlorinated and chloraminated samples. In general, monochloramine does not reduce the occurrence but may have an effect on the concentration of *L. pneumophila* Sg1 (Figure 4). The spread of median concentrations of *L. pneumophila* Sg1 per tap showed that concentrations in water treated with monochloramine had a lower variability. Also, the 75th percentile monochloramine value is a full log lower than that for the chlorine treatment. Whether these trends reflect real differences caused by disinfection treatment type will require studies with greater statistical power. However, the data suggest that monochloramine may be a better disinfectant for *L. pneumophila* Sg1 in potable water. In Figure 4, 75% of the samples from monochloraminated water were well below the LD for culture. This suggests that the significant drop in detection frequency identified in studies by Moore et al. (2006) and Flannery et al. (2006) was due to the concentration of *L. pneumophila* Sg1 dropping below the LD for culture. Partial evidence to support this hypothesis is found in the report by Wang et al. (2012). They revisited some of the sites studied by Moore et al. (2006) and detected *L. pneumophila* in 5.6% of the water samples and at 20% of the sites using a qPCR technique compared to the 6.2% of sites reported by Moore et al. (2006). Unfortunately, Wang et al. (2012) only reported the highest concentration and the average concentration for water samples making it difficult to determine the percentage of samples with concentration below the culture LD.

Overall, *L. pneumophila* Sg1 was molecularly detected in 19.5% (18/92) of the water samples from this study disinfected with monochloramine. This frequency of detection is larger than what Wang et al. (2012) reported for both their monochloramine locations. This is most likely due to the broad geographic area and multiple sampling episodes covered by this study.

In our study, two qPCR assays were evaluated and found suitable for the detection and quantification of *L. pneumophila* and *L. pneumophila* Sg1 in potable water. Over two years, 68 taps were each monitored four times. *L. pneumophila* Sg1 was detected in 47% of the taps. In the majority (31%) of cases, only one sample from a given site tested positive. There were a total of 11 taps that showed colonization, but only five reached concentrations that exceeded $a \times 10^5$ GU/L qPCR action level for *L. pneumophila* Sg1 as suggested by Lee et al. (2011).

This study looked at *L. pneumophila* Sg1 occurrence on a limited but national scale. It provided evidence that exposure to *L. pneumophila* Sg1, the species/serogroup etiologic agent attributed to most legionellosis cases, occurs at point of use taps that provide potable water to workers and families. This study demonstrates that home and facility water distribution lines can become a vehicle for exposure to *Legionella* when aerosolized.

Over the past decade, the incidence of legionellosis has increased in some areas across the US, especially the Northeast/Middle Atlantic regions. Collier et al. estimated that 433 million dollar per year is spent for the hospitalization of legionellosis cases. The present study demonstrated that gene products of *L. pneumophila* Sg1 are occurring and colonizing at point of use taps at locations across the U.S. Further research is needed to identify biotic and/or abiotic factors that contributed to *L. pneumophila* Sg1 occurrence at the locations evaluated.

### ASSOCIATED CONTENT

Supporting Information

Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

The authors declare no competing financial interest.

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### REFERENCES


