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Protocol for responding to the detection of *Legionella pneumophila* in drinking water distribution systems

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Abstract

A protocol for responding to *Legionella pneumophila* detections in distribution system samples is presented and justified. The protocol was developed using existing protocols for building water systems, vetted in workshops, and provided to utilities participating in a research project. The protocol provides action levels and actions that utilities can take when *L. pneumophila* is detected in distribution system samples. Action levels are based on the best available science and expert judgment and are similar to those in protocols for assessing building water system monitoring results. Action levels should be reassessed as additional data and knowledge on the occurrence and growth potential of *L. pneumophila* in distribution systems become available. All positive detections trigger assessments that focus first on conditions local to the sample location and then extend further into the distribution system. Higher concentration or more frequent detections initiate more assertive responses and communication than lower concentration and frequency detections. The protocol provides a starting point for the development of protocols for purposes such as *L. pneumophila* operational surveillance or within an outbreak investigation.

KEYWORDS

distribution systems, *Legionella*, microbial monitoring, microbial water quality, public water systems, water safety planning

1 | INTRODUCTION

A protocol for responding to positive *Legionella pneumophila* samples collected from the distribution system (DS) of public water systems (PWSs) is a prerequisite for monitoring, whether within a research project, for remediation or disease outbreak follow-up, for process control, or potentially, for regulatory compliance. In this article, we present a protocol that was developed for responding to positive DS *L. pneumophila* samples for a research project. The protocol was developed prior to the start of DS sampling, vetted at an experts' workshop, and distributed as a resource to utilities participating in the study. This article presents background information used to formulate the protocol, summarizes results from the experts' workshop, and presents the protocol used as guidance in the *L. pneumophila* research project. Although this project focused on *L. pneumophila* as the waterborne pathogen of greatest present concern to the CDC (Barskey et al., 2022), *L. pneumophila* is only one of the opportunistic premise plumbing pathogens (environmental organisms common in potable water systems and that pose elevated risks to susceptible individuals) that are an emerging concern within the drinking water community (Collier et al., 2021) and that remain a research need.

At present, there is no definitive guidance from the United States Environmental Protection Agency (EPA) nor the US Centers for Disease Control (CDC) on the interpretation of levels of *Legionella* in PWS DS water. However, several national and international guidelines, described below, provide guidance on how to interpret and respond to the detection of *Legionella* in building water piped systems. Although these guidelines are intended for building water systems, their underlying risk management strategies can be applied to the DS of a PWS. Relevant protocols are summarized below and reviewed in greater depth in Supplementary Materials. Other standards and guidance for building water systems (ASHRAE, 2000, 2018, 2023; Centers for Medicare & Medicaid Services (CMS), 2017) include requirements or recommendations for monitoring, but do not include specific recommendations on action levels or actions for positive samples and are not reviewed.

New York State regulations require hospitals and health care facilities to monitor their potable water systems for culturable *Legionella* and institute control measures and notify authorities when 30% or more of the potable water samples contain *Legionella* spp. (Public Health Law, 2016). The 30% action level is problematic because it might not be exceeded even for a system with widespread, uncontrolled contamination and because it does not consider the concentration of *Legionella* in individual samples. Low levels of *Legionella* are possible for a system that is properly managing *Legionella*, whereas high levels always indicate a loss of control and amplification of the organism. Revised Allegheny County guidelines for *Legionella* management (Moore & Shelton, 2014) acknowledge that the 30% target has limited scientific support and is problematic, but do not propose a specific alternative assessment.

The American Industrial Hygiene Association (AIHA) provides guidelines for routine *Legionella* monitoring in the absence of any reported illnesses (AIHA, 2022). AIHA guidelines specify action levels for incoming water (typically from a PWS), water from samples in the potable water DS (inclusive of hot water distribution), and non-potable water system components such as cooling towers. Guidelines for interpreting and responding to results from supply water sampling are presented in Table 1. In contrast to the New York State action level, the AIHA assessment considers the *Legionella* level, but not the extent or duration of contamination (the proportion of positive sites or repeat positives in successive sample rounds at a given site).

Under the Surface Water Treatment Rule (SWTR), EPA regulates all *Legionella* species with a non-enforceable Maximum Contaminant Level Goal (MCLG) of zero. Similarly, the New York State monitoring requirements and Allegheny County guidelines specify monitoring for *Legionella* spp. However, the currently available culture media does not recover all species equally. The genus *Legionella* is comprised of >60 species, consisting of 70 serogroups, and the culture media originally developed to isolate *L. pneumophila* are not equally effective for recovery of all *Legionella* species (Descours et al., 2014; Lee et al., 1993; Lück et al., 2004). Johnson et al. (2018) detected a wide range of *Legionella* species (16 different species in total) in reclaimed water by qPCR, but 96% of the isolates recovered on BCYE agar were *L. pneumophila*. So, there is little value in specifying culture methods for the whole genus *Legionella* when current methods largely target *L. pneumophila*. Moreover, there is no scientific basis for equating counts of *L. pneumophila* to other species of *Legionella* due to the differences in virulence and clinical outcomes. Because qPCR techniques cannot determine the viability of detected isolates, the AIHA guideline specifies analysis of *Legionella* by culture (to determine the effectiveness of control strategies) and recommends that PCR techniques be used as a complementary analysis but that they should not replace culture-based methods.

The European ESCMID Study Group for *Legionella* Infections (ESGLI) published proposed guidelines for the prevention, control, and investigation of infections caused by *Legionella* species in hot and cold potable building water systems (European Guidelines Working Group, 2017). The guidelines emphasize proper water management plans focusing on obtaining proper temperature, biocide, and operation and maintenance programs. Monitoring is performed to demonstrate the effectiveness of the plans (not to detect conditions posing unacceptable acute risk). These goals incorporate both numerical threshold values, such as in the AIHA guidelines, along with targets for frequency of exposure—such as in the New York State and Allegheny County guidelines. The ESGLI guideline emphasizes the goal to achieve no culturable *Legionella* but acknowledges that occasional detection (<20%) of low levels of *Legionella* (<1 CFU/mL) may be acceptable provided that other water quality values (e.g., water temperature, residual disinfectant, etc.) and operational parameters were within the water

Article Impact Statement

This study's positive *Legionella pneumophila* detection response protocol was used in a research study, may be adapted by other utilities, and can advance utility adoption of *L. pneumophila* monitoring.

TABLE 1 AIHA action levels and actions for potable water systems (excerpted from AIHA, 2022).

	No action	Potential for growth		Possible growth		Indicates growth	
Sample source	Level (CFU/mL)	Level (CFU/mL)	Action	Level (CFU/mL)	Action	Level (CFU/mL)	Action
Potable water (incoming municipal water)	Non-detectable	<1	A	1 to <10	B	>10	C
A: Monitor. Measure and document incoming water							
B: Investigate and mitigate risks of growth 1. Measure and document incoming water temperature, disinfectant levels, and pH every other day (1–2 weeks). 2. Investigate possible causes of water service disruption or disturbance, such as water main or service line breaks, and/or nearby construction that might be dislodging deposited sediment, debris, or corrosion. 3. Notify municipal water supplier of findings and request investigation of contributing factors. If low disinfectant levels are determined to be an issue, implement measures to increase them. 4. Based on professional judgment and the history of the water system, consider increasing the frequency and/or scope of sampling efforts in the premise plumbing in order to identify high-risk sites of amplification source, such as water heaters or low-use areas. If disinfectant levels are increased, re-test the incoming water for culturable *Legionella* after 1–2 months							
C: Investigate, mitigate risks of growth, and enhance control measures 1. Measure and document incoming water temperature, disinfectant levels, and pH every other day (1–2 weeks). 2. Notify municipal water supplier of these findings and request investigation of contributing factors. If low disinfectant levels are determined to be an issue, consider supplemental disinfectant. 3. IMMEDIATELY examine secondary parameters (pH, residual disinfectant levels, water temperature, etc.) in the premise plumbing to identify potential effects of elevated *Legionella* levels in the municipal water supply. 4. Carry out a complete *Legionella* source assessment for at-risk premise plumbing and other building water systems that receive water from this service. Take appropriate actions based on the findings of the building water system assessment. 5. Based on professional judgment and the history of the water source, consider increasing the frequency and/or scope of sampling efforts in the premise plumbing in order to identify high-risk sites of amplification source, such as water heaters or low use areas. 6. Re-test the incoming water for *Legionella* after 1 month.							

management plan guidelines. Intermediate levels (1–10 CFU/mL) and high levels (>10 CFU/mL) would trigger a series of actions including resampling, onsite disinfection, and overall review of the water management plan program. Triggering these remedial actions would result in prompt actions to protect public health—consistent with the qualitative microbial risk assessment (QMRA) results of Hamilton et al. (2019)—while still providing flexibility for building owners and water systems to deal with low, sporadic detections of *Legionella* in water.

The CDC has developed a toolkit with action levels and responses for *Legionella* management and control in building water systems (CDC, 2017, 2021). The CDC protocol employs a multi-tiered assessment approach that uses both *Legionella* spp. culture monitoring data and other water system operational data to assess the level of *Legionella* control (well controlled, poorly controlled, or uncontrolled). Control is assessed based on individual sample concentrations, changes in concentration over time, *Legionell*a species detected, and extent of occurrence in a given sample round. Generally, *Legionella* is considered well controlled at concentrations that range from detectable to 0.9 CFU/mL and are detected at only a few locations on a given sample round. Full details of the CDC multi-tiered approach are presented in supplemental materials.

Generally, research studies that monitor for *Legionella* spp. and *L. pneumophila* via molecular methods report very high occurrences in DS water and sediment samples (Hu et al., 2021; Isaac & Sherchan, 2020; Lu et al., 2015, 2016; Perrin et al., 2019; Zhang et al., 2021). Features such as low residual disinfectant concentration, high water age, and presence of unlined cast iron pipe are associated with higher occurrence and concentrations of legionellae. Research studies using culture methods (Gleason et al., 2023; LeChevallier, 2019a, 2019b; Omoregie et al., 2022) report a far lower occurrence of legionellae, higher occurrence for warm waters with low to no residual disinfectant, and, when legionellae are detected, low concentrations relative to the concentrations associated with a significant risk of illness. A particularly important finding of LeChevallier (2019a, 2019b) is that there is a marked difference in the detection and abundance of *L. pneumophila* (measured via culture method) between water with very low or no residual disinfectant and water with measurable and effective residual disinfectant, but that *L. pneumophila* can still be

detected in low concentrations in water with high residual disinfectant concentrations. This finding indicates that monitoring for residual disinfectant and control parameters, but not *L. pneumophila*, might not be sufficient for assuring sufficient controls are in place and that the residual disinfectant is not the critical barrier, but only one of multiple barriers that must be in place within a comprehensive *Legionella* control program.

The action levels used by the AIHA, CDC, and in the 2017 European technical guidelines are significantly below those associated with a significant (e.g., 10^{-4}) risk of Legionnaires' disease. In their QMRA study of *L. pneumophila* exposure through aerosols from showers, faucets, and toilets for both single exposure events and as an annual risk, Hamilton et al. (2019) estimated a risk of illness of 10^{-4} for a single shower event in water with a steady *L. pneumophila* concentration of 3.5×10^5 CFU/L (350 CFU/mL). Recently, the National Academy of Sciences, Engineering and Medicine (NASEM) reviewed studies of legionellosis sporadic cases and outbreaks and, based on environmental monitoring data presented in those studies, proposed that a *Legionella* concentration of 50,000/L (or 50/mL) should be considered as an "action level" high enough to warrant immediate remediation (National Academies of Sciences, Engineering, and Medicine, 2019), although the report cautioned that "a lower action level may be necessary to protect those at higher risk for legionellosis such as hospital patients, particularly those in intensive care, cancer, and solid-organ transplant units." Similar to the level estimated by Hamilton et al. (2019), the NASEM level is referenced to acute human health risk, not *Legionella* control. Because the Hamilton and NASEM levels are well above those used by AIHA, European Union, and CDC for assessing control, they support the action levels as both protective of human health and useful for application within a water management program for *Legionella* control.

This article presents a protocol developed for a research project. The protocol was developed to prepare utilities participating in the project to respond to positive *L. pneumophila* detections in samples from their DSs. The project, described in greater detail below, entailed utility staff collection of DS samples and analysis using the Legiolert assay (Legiolert®; Idexx laboratories Inc, Westbrook, ME). Legiolert® is a culture method that detects *L. pneumophila* (not *Legionella* spp.). It is an MPN (most probable number) method and results are typically reported as MPN/100 mL because, for most potable water applications, the sample volume assayed is 100 mL. Although the protocol reported in this study was developed specific to *L. pneumophila* detections via Legiolert®, the protocol could be revised for use with other culture methods and with *Legionella* spp. as the target.

2 | MATERIALS AND METHODS

2.1 | Research project

The protocol reported in this article was developed within a larger research project whose objectives include: (i) developing a response and communication protocol that utilities can use following positive *L. pneumophila* detections; (ii) collecting data to characterize the relationship between *L. pneumophila* occurrence/concentration and disinfectant residuals in US PWS DSs; (iii) identifying locational characteristics associated with higher likelihood of *L. pneumophila* occurrence; and (iv) conducting analyses to quantify the association between disinfectant residual (type and concentration), utility characteristics, and occurrence and abundance of *L. pneumophila* in bulk water samples.

Fifty utilities were recruited to sample weekly for 12 weeks over two summers for *L. pneumophila*. Participating utilities collected samples from at least three locations with conditions favoring *L. pneumophila* presence and when water temperature exceeded 18°C. Most of the participating utilities analyzed samples in-house using the Legiolert method. Positive Legiolert® quantitrays were sent to a partner laboratory where contents of positive wells were subject to analysis via ISO method 11731:2017 such that colonies could be collected, sequenced, and subjected to additional analyses. Sample collection was scheduled for completion before November 2023.

2.2 | Workshops

Two workshops were conducted to elicit subject matter expert input on a draft protocol for responding to positive DS *L. pneumophila* samples. The first was held on May 24, 2022, and attended by more than 50 experts in drinking water including representatives from water utilities, regulatory agencies, academic institutions, consulting companies, and professional organizations. A draft response protocol for use by utilities conducting DS monitoring as part of an ongoing research project was distributed for review along with additional related information prior to the workshop. The primary goal of the workshop was solicitation of comments and recommendations from participants for the protocol as a part of the ongoing research project. Although outside the scope of the research project, a secondary goal was solicitation of comments and suggestions for extending the protocol to be general guidance for utilities conducting routine *L. pneumophila* monitoring.

Generally, participants were supportive of the draft protocol and agreed with the project team's assessment

that development of a protocol that is acceptable to utilities, regulators, and other stakeholders is a critical enabling step for non-regulatory *L. pneumophila* monitoring. Important discussions and comments during the workshop highlighted the following:

- The importance of establishing a relationship between participating utilities and their regulators prior to initiating monitoring.
- That routine monitoring is already underway by utilities in some states and that some sample results are already provided to regulatory authorities.
- Monitoring identifies locations and conditions under which *L. pneumophila* is not adequately controlled.
- There are multiple water quality benefits of instituting corrective actions in response to positive detections.

Based on workshop and internal project team discussions, the draft protocol was modified and distributed to utilities participating in the *L. pneumophila* occurrence survey research study. The protocol presented in this study is the modified version with additional modifications, clarifications and improvements suggested by peer-reviewers during review of this article for publication in Water Science.

At the request of Association of State Drinking Water Administrators (ASDWA), a follow-up workshop on responses to positive detections was conducted on July 13, 2022. The workshop started with a condensed version of the background information presented at the 24 May workshop and the event was structured to encourage participants to comment on the proposed approach. Questions from participants generally were for clarifications on the proposed protocol and to consider additional utilities for participation in the ongoing research effort. Together, the workshops served their intended purpose of eliciting stakeholder input and vetting and improving a response protocol for use in the research project. The topic with the greatest diversity of opinions during the workshops was reporting and communication responsibilities associated with positive *L. pneumophila* detections. Stakeholders took positions ranging from all positive samples should be reported to state regulatory agencies, irrespective of the concentration, to no results should be reported because there are no current national regulations for legionellae in DSs. The protocol described in this manuscript was developed specifically for the larger research effort and establishing communication and reporting responsibilities was outside the project scope. Nonetheless, consensus on reporting and communications related to *L. pneumophila* detections could benefit both regulators and regulated utilities, and further review and development of a protocol acceptable at the national scale should be a research and policy development focus.

3 | RESULTS

3.1 | Protocol

A flowchart showing the recommended response to a positive *L. pneumophila* detection, in an initial sample and its repeat sample, is presented in Figure 1 and draft trigger levels and actions are embedded in the response as presented in Table 2. Although the sequence presented in Figure 1 was developed for a research project, with modification it could be used for routine monitoring as a component of a *L. pneumophila* risk management program. Rows assuming collection of 10 or more samples were not applicable for the research project, since participating utilities typically collected only three samples per sample round.

The broad steps in the protocol are: (A) microbial method and sample collection QA/QC; (B) follow-up analyses; (C) repeat sample; and (D) actions to take based on results.

Sample collection and microbial analysis QA/QC checks (A) are similar to those used for any microbial analysis (e.g., total coliform sampling and analysis). Because *L. pneumophila* are common members of the building microbiome and because samples might be collected from connected buildings rather than dedicated sample stations, sample collection procedures (especially adequate flushing of the sample tap) must be reviewed carefully for each positive sample. If QA/QC checks (A) fail, utilities resample within 24 h from identification of the QA/QC failure.

Follow-up microbial analyses (B) is not resampling; it is additional analysis of positive samples and can be conducted for multiple purposes. In the research project, contents of positive Legiolert® trays were subject to follow-up analysis via ISO Method 11731:2017 and qPCR. This additional step was not strictly necessary and was taken to enable collection of *L. pneumophila* isolates for sequencing. A recent study (Matthews et al., 2022) found that *L. pneumophila* isolates can also be obtained directly from the Legiolert® tray for serotyping and/or storage for a year or more. If ISO Method 11731:2017 is used instead of Legiolert®, follow-up analyses of positive samples should identify organisms to the species level and serogroup for *L. pneumophila* isolates. In our research study, if the follow-up analysis failed to detect *L. pneumophila* in the sample (this occurred for less than 4% of positive samples), the result was considered negative and no further action was taken. Current research is comparing

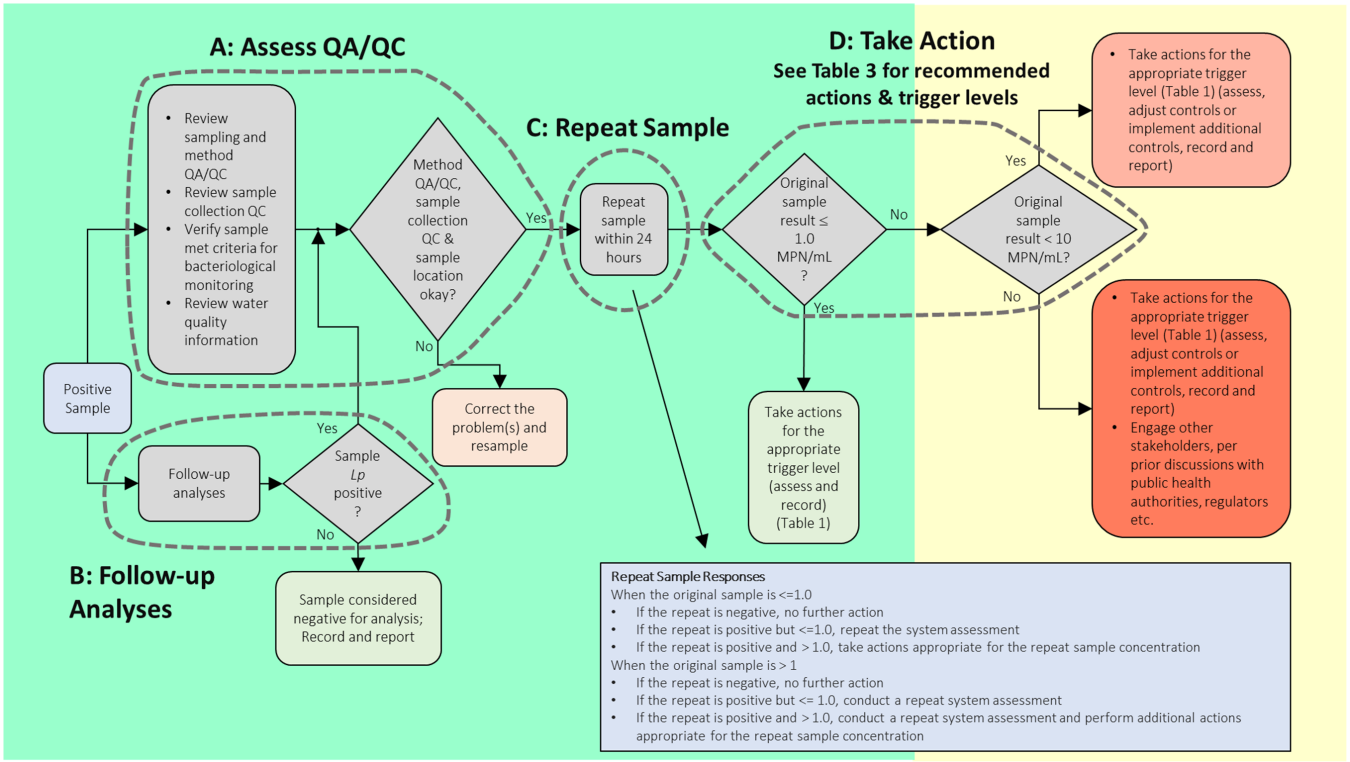

FIGURE 1 Culture-based positive detection of *L. pneumophila*: A flowchart guiding follow-up evaluation (to be used in combination with Table 2).

subculturing using ISO Method 11731:2017 or qPCR, and other serotyping techniques to determine the optimum procedure for *L. pneumophila* recovery from Legiolert® trays. If a monitoring effort used the ISO method instead of Legiolert®, a follow-up analysis would be required to confirm the presence of *Legionella* spp. and the species. If QA/QC problems are identified, the sample is invalidated and the site is resampled. If no QA/QC problems are identified, a repeat sample is collected within 24 h of completing the QA/QC (C) and the sample analysis and QA/QC processes are repeated (steps A and B). Note that the repeat sample is often collected before all the follow-on *L. pneumophila* recovery is completed.

The specific actions (D) to be taken in response to a *L. pneumophila* detection depend upon the concentration of *L. pneumophila* in the original positive sample and the repeat sample. These actions are outlined in Table 2. Actions and communication for each combination of concentration and additional factors should be conducted. For example, if a sample for a site is in the range 1.0 to ≤10 MPN/mL and the site is positive in two successive samples and disinfectant concentration is below the control limit, actions and communications specified in all applicable rows should be conducted. Note that *L. pneumophila* concentrations in Table 2 have units of MPN/mL because Legiolert® is an MPN method. Because MPN and CFU are equivalent measures, action level units would be CFU/mL if an alternative culture method was used. If the original *L. pneumophila* concentration in the sample is low, and the repeat sample is negative (which is the most common result), and the system water quality is within normal operating ranges, the results are noted and routine sampling continues. However, if *L. pneumophila* concentrations are higher and occur more frequently, then additional corrective actions including notification of regulatory authorities would be recommended, as specified in Table 2. Note that assessments based on the proportion of positive samples only apply when more than 10 samples are collected in a sample round or when the assessment is made based on data pooled from multiple sample rounds. System assessments are conducted for all positive samples without waiting for repeat sample results. Additional actions (described in Table 2) are taken when the original sample concentration is ≥10 MPN/L. If this protocol is revised for use beyond the research project, action levels should be reassessed as additional data and knowledge on the occurrence and growth potential of *L. pneumophila* in DSs become available.

Whether for a research project or for other purposes, additional contextual data are required for full use of

TABLE 2 Action levels and responses.

L. pnuemophila **concentration**	**Additional factors**	**Actions per *L. pneumophila* concentration and additional factors**	**Communication and notification**
<0.1 to 1.0 MPN/mL		• Assure water quality values are within target range and have not changed significantly from operational goals. • Check residual disinfectant for recent changes or low values.	• Record results and report to water quality manager.
>1.0 to ≤10 MPN/mL	<20%. This assumes that 10 or more samples are collected per sample round	• Resample positive locations within 24 h. • Consider whether results indicate a section of the distribution system is experiencing positive detections or positive detections are occurring randomly throughout the system. Conduct a system assessment based on the findings. • Continue to track ongoing water quality and *L. pneumophila* data for changes.	• Record results (including resampled results) and report to water quality manager; document the system review. Continue to track ongoing water quality data along with *L. pneumophila* results. Per prior arrangements with the regulatory authority, report results to the regulatory authority.
	≥ 20% of samples are positive; This assumes that 10 or more samples are collected per sample round	• Resample positive locations within 24 h. • Conduct a broad system assessment and evaluate options for mitigative controls which could include storage facility inspection and cleaning, boosting the total chlorine residual, unidirectional flushing, and so forth. • Evaluate trends in system water quality and pressure.	• Record results (including resampled results) and document responses. Report to water quality manager, upper management. Per prior arrangements with the regulatory authority, report results to the regulatory authority.
	Location positive in successive routine samples	• Resample positive locations within 24 h. • Carefully review conditions around the sample location for tap associated conditions, building associated conditions, service line conditions, and supply main conditions.	• Record results (including resampled results) and report to water quality manager; document the investigation of local conditions. Per prior arrangements with the regulatory authority, report results to the regulatory authority.
	Water quality parameters or other conditions outside control limits	• Conduct a system assessment to determine the reason for excursion outside control limits. • Update control measures as needed.	• Record results (including resample results) and report to water quality manager; document the system assessment.
>10 to <50[a] MPN/mL		• Resample positive locations within 24 h[b] • While waiting for resample results, conduct an immediate review of control measures.	• Record results (including resampled results) and document responses. Report to water quality manager, upper management. Per prior

(Continues)

TABLE 2 (Continued)

L. pnuemophila concentration	Additional factors	Actions per *L. pneumophila* concentration and additional factors	Communication and notification
		• Conduct corrective actions such as flushing, valve check and increasing disinfectant residual if possible and warranted. • If repeat sample is <10 MPN/mL, take a second repeat sample; if repeat sample is ≥10 MPN/mL, take appropriate actions. • For additional repeated positive samples, continue system assessment with a goal to identify the deficiency and appropriate corrective actions	arrangements with the regulatory authority, report results to the regulatory authority.
≥50[a] MPN/mL		• Resample positive locations within 24 h. • While waiting for resample results, conduct an immediate review of control measures. • Conduct corrective actions such as flushing, valve check and increasing disinfectant residual if possible and warranted. • If repeat sample is <10 MPN/mL, take a second repeat sample; if repeat sample is ≥10 MPN/mL, take appropriate actions. • For additional repeated positive samples, continue system assessment with a goal to identify the deficiency and appropriate corrective actions	• Record results (including resampled results) and document responses. Report to water quality manager, upper management. Per prior arrangements with the regulatory authority, report results to the regulatory authority.

Note: The actions and communication are to be conducted according to the *L. pneumophila* concentration along with the corresponding additional factors. The following actions are to be followed in concert with the flowchart (Figure 1).

[a]Value is based on NASEM analysis of outbreaks. This could be replaced with an alternative, appropriate risk-based target. For example, this could be the concentration with a 5th percentile risk estimate of 1×10^{-4} risk of illness per exposure (690 F U/mL, per Hamilton et al., 2019).

[b]Flushing is recommended as a first step particularly when disinfectant concentration is low (<0.2 mg/L). If the sample positive associates with a storage facility, consider draining, cleaning, and disinfecting the facility.

L. pneumophila data. Critical contextual data that were collected for the current research study include exact location of sample collection, characteristics of the sample site (e.g., is the sample from a tap in a building and potentially subject to changes from the building plumbing system?), time and date of collection, time and date of sample processing and results, analytical results, quality control data, disinfectant residual concentration, water temperature, pH, and possibly other water quality data such as heterotrophic place count (HPC) and turbidity. Other important contextual data include any report of recent nearby construction, hydrant flushing, main break, valve operation, storage facility conditions, or other activities that could contribute to an increase in *L. pneumophila* or disrupt biofilm such as with changes in the direction of water flow or water pressure transients. The conditions of sample collection and the sample tap location can be very important for understanding the sample's analysis results. This has been shown with sampling for total coliform and *Escherichia coli* (Dufresne et al., 1997). Sample taps should be representative of the water in the DS rather than in the plumbing of the building or the service line, or the hydrant barrel and supply line if a hydrant is used. Sample locations should be

adequately flushed (e.g., 3–5 min), reviewed periodically to determine whether they have higher rates of positive samples, and maintained in a sanitary condition.

3.2 | System assessments

Systems assessments are recommended as follow-on actions for samples with a *L. pneumophila* concentration >1 MPN/mL. Those system assessments are likely to follow guidance for responding to positive coliform and *E. coli* results under the US EPA Revised Total Coliform Rule (RTCR). Under the RTCR, based on the occurrence of coliform bacteria or *E. coli*, two levels of assessments might be required: Level 1 and Level 2. The Level 1 assessment is a self-assessment, looking for obvious factors and conditions that could lead to positive samples. A Level 2 assessment expands this, requiring more experienced assessors and a broader, more extensive review of system conditions. Since water utilities under the EPA's RTCR, as well as their enforcement agencies, should be familiar with these assessments, they provide a good strategy for response to *L. pneumophila* positive samples. Guidelines and considerations for those assessments are presented below. For detections at higher levels, some regulatory authorities might recommend or require Level 2 assessments as an appropriate response.

System assessments can be conducted for all or part of the DS. Generally, assessments start locally and broaden out if nothing is found. An assessment would start with evaluation of laboratory quality control and an evaluation of the conditions of the sampling location (such as a check for unsanitary sample taps, cross connections, and leaking service meters in meter pits), broaden to the DS (such as a check for closed valves or stagnant water mains, a check for local main breaks and repairs as well as flushing events and rusty water occurrences, and a check for flow reversals and disturbances), broaden outward to storage facilities and pressure districts, and eventually, if needed, back to the water treatment plant and source water. If greater than 20% of samples taken within a sampling period for the whole system or for a pressure district are confirmed positive, then the water utility should begin looking at system-wide conditions. The 20% target was set to be consistent with targets set for building water systems and should be reduced as additional data are collected that allow a more refined estimate of the occurrence of *L. pneumophila* occurrence in well-run DSs.

A system assessment starts locally and broadens as appropriate and can entail the following general tasks:

- Identifying conditions or events that could promote *L. pneumophila* occurrence, displacement, and growth. Most often, such conditions are simultaneous occurrence of some combination of low residual disinfectant concentration (<0.2 mg/L) and warm water temperature (>18°C), flow changes and disruptions (e.g., change of valve position), and loss of DS integrity or regrowth in a storage facility. Examples of associated events are main breaks and low-pressure transients.
- Evaluating whether the associated conditions and/or events may have caused the *L. pneumophila* occurrence. Note that it may be impossible to prove that certain conditions, especially if they are transient, caused a *L. pneumophila* occurrence.
- Identifying whether controls are in place and active to manage *L. pneumophila* growth.
- Evaluating whether controls are sufficient to manage *L. pneumophila* growth. If the controls are insufficient, they should be evaluated and adjusted.

3.3 | Communication

Communication strategies and tools should be in place prior to the initiation of sampling. A recent research project report (Masters et al., 2018) outlines materials that water utilities can use to proactively communicate with customers about *L. pneumophila* in water. Included in the messaging is the opportunity to communicate with customers about what the water utility is doing to manage *L. pneumophila*. Outreach and educational materials provide an opportunity for water utilities to explain why they maintain a disinfectant residual, what other safeguards and barriers they have in place for *L. pneumophila* management, the necessity for infrastructure renewal, and the importance of regular cleaning and flushing of the DS. They also afford an opportunity to emphasize the shared responsibility for *L. pneumophila* management with customers who operate building water systems. For routine monitoring, development of communication strategies (who, when, how, and what) will require close consultation of utilities, regulators, public health authorities, and subject matter experts and will likely differ from state to state and perhaps utility to utility. Close collaboration between all stakeholders can produce strategies that are protective of human health without causing undue concern or unnecessarily eroding the public's confidence in their drinking water supply.

Within the research project, the following levels of communications were recommended:

- For results ≤1.0 MPN/mL and where disinfectant residuals and other operational parameters are normal and when repeat samples are negative, no further communication is required as these results indicate normal

background occurrence. Regulators and public health authorities can be periodically updated on the results (as per prior agreement).

- For results >1.0 and ≤10 MPN/mL, the utility should take proactive steps to flush, disinfect, and remediate conditions that might have contributed to the *L. pneumophila* results. Follow-up samples should validate the effectiveness of the actions. If requested in prior discussions with regulators, regulators and public health authorities should be informed of these actions.
- For results >10 MPN/mL, and particularly >50 MPN/mL, the results should be reported to the regulators and public health authorities and consultations should be held to determine the appropriate responses and types of notifications. To date such levels have not been encountered in properly managed DSs.

Some US regulatory authorities require a higher level of communication; in those cases, the applicable regulations should determine the communication levels. This should be clearly laid out in advance between the water utility who will be doing the monitoring and its regulatory agency or state.

4 | DISCUSSION

L. pneumophila occurrence in PWS DSs and the relationship between *L. pneumophila* occurrence and system factors (water quality, system characteristics, system operation) is less studied than for other piped water systems such as cooling towers and building plumbing systems (National Academies of Sciences, Engineering, and Medicine, 2019) and remain a critical data gap and obstacle to informed regulation and management of *L. pneumophila* in PWSs. Filling those data gaps requires research involving *L. pneumophila* monitoring which, in turn, requires protocols in place prior to monitoring for responding to positive samples. Having protocols in place prior to initiating research improves the likelihood that utility responses to detections during the research monitoring will be effective, timely, and coordinated with public health authorities and potentially impacted customers.

This study presents a protocol developed for water utilities participating in a large, US national-scale survey of *L. pneumophila* in DSs and the factors associated with their occurrence. Because some utilities already conduct routine *L. pneumophila* monitoring and others are likely to start in the near future, the protocol presented in this article can also form the basis for general guidance for use by both utilities and regulatory agencies to promote better and more effective *L. pneumophila* monitoring and water quality management. Ultimately, regulatory authorities will determine the requirements, if any, for ongoing monitoring as well as for any research project.

Adapting this protocol for wider use could promote increased routine *L. pneumophila* monitoring as an element of water quality management among utilities. Every governmental, professional association, and working group that has considered *Legionella* risk management in buildings has concluded that a Water Management Program (WMP) or Water Safety Planning (WSP) approach is the most actionable and effective approach for *Legionella* control. The WMP/WSP approaches require identifying hazardous conditions, enacting controls where required, monitoring the controls, and monitoring *Legionella* to confirm that the controls are adequate. This same approach can be applied for *L. pneumophila* management by drinking water utilities/suppliers (Burlingame & Bartrand, 2023).

Necessary elements for implementation of WMP/WSP approaches to *L. pneumophila* control are analysis methods, which may be used practically by utilities and that produce actionable data and criteria for assessing and acting upon results from monitoring. At present, culture analysis techniques are the only option that yields *L. pneumophila* concentrations, which may be compared against action levels. Legiolert® has emerged as a viable analysis method that may be implemented at the utility level and with sensitivity consistent with action levels proposed in this study and in other protocols for responding to positive samples (Barrette, 2019; Boczek et al., 2021; Checa et al., 2021; Dowdell et al., 2022; Hirsh et al., 2021; Inoue et al., 2020; LeChevallier, 2019a, 2019b; Mapili et al., 2020; Maqbool, 2019; McCuin et al., 2021; Niu et al., 2022; Omoregie et al., 2022; Petrisek & Hall, 2017; Rech et al., 2018; Sartory et al., 2017; Scaturro et al., 2020; Spies et al., 2018). Because most qPCR assays do not distinguish between genetic material from live and dead organisms, surveys with qPCR indicate very high occurrence (proportion positive samples) of *Legionella*, making the results less useful in diagnosing and controlling *Legionella* growth. qPCR surveys of *Legionella* in DSs and building water systems (Ditommaso et al., 2015; Waak et al., 2018; Whiley et al., 2014) have demonstrated that data from molecular methods are indicative of process control and point to the potential for using qPCR *Legionella* data as a biological water quality indicator (Zhang & Lu, 2021). Research is required to develop action levels for interpreting and using qPCR data. Additional potential applications of qPCR within a *Legionella* spp. or *L. pneumophila* monitoring framework include use as a screening step (only qPCR positive samples for the target are subject to culture analysis) or to develop more specific information about legionellae recovered from culture assays (e.g., serotyping or sequencing).

Workshop discussions indicated that regulators are likely to differ in the level of information they request/require following a positive *L. pneumophila* detection. At least one US regulatory authority already requests reporting of all *L. pneumophila* positive samples whereas others do not require results for unregulated organisms/contaminants or for research projects. Given this reality, utilities undertaking *L. pneumophila* monitoring for any purpose should coordinate response and communication with regulatory authorities prior to initiating sampling. Both regulatory and regulated communities would be well served by further discussions and development of testing protocols that protect public health and afford utilities the opportunity to understand and manage *L. pneumophila* in their DSs.

AUTHOR CONTRIBUTIONS

Timothy A. Bartrand: Conceptualization; funding acquisition; writing – original draft; project administration. **Mark W. LeChevallier:** Conceptualization; methodology; writing – review and editing. **Jennifer L. Clancy:** Conceptualization; methodology; writing – review and editing. **Gary A. Burlingame:** Methodology; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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SUPPORTING INFORMATION

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

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