Supplementary Information (SI)

Legionella pneumophila occurrence in reduced-occupancy buildings in 11 cities during the COVID-19 pandemic

Authors

Katherine S. Dowdell[1,**] and Hannah Greenwald Healy[2,**], Sayalee Joshi[3,4], Marianne Grimard-Conea[5], Sarah Pitell[6], Yang Song[7], Christian Ley[8,13], Lauren C. Kennedy[2,9], Solize Vosloo[16], Linxuan Huo[10], Sarah-Jane Haig[6,11], Kerry A. Hamilton[3,4], Kara L. Nelson[2], Ameet Pinto[10], Michele Prévost[5], Caitlin R. Proctor[12,13] , Lutgarde M. Raskin[1], Andrew J. Whelton[13,14], Emily Garner[15,*], Kelsey J. Pieper[16,*], and William J. Rhoads[17,*]

1. Department of Civil and Environmental Engineering, University of Michigan, Ann Arbor, MI, USA.
2. Department of Civil and Environmental Engineering, University of California, Berkeley, Berkeley, CA, USA.
3. School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, AZ.
4. The Biodesign Center for Environmental Health Engineering, Arizona State University, Tempe, AZ.
5. Department of Civil, Geological and Mining Engineering, Polytechnique Montreal, Montreal, QC, CAN.
6. Department of Civil and Environmental Engineering, University of Pittsburgh, Pittsburgh, PA, USA.
7. Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.
8. Department of Civil, Environmental, and Architectural Engineering, University of Colorado - Boulder, Boulder, CO, USA.
9. Department of Civil and Environmental Engineering, Stanford, Palo Alto, CA, USA.
10. Department of Civil and Environmental Engineering, Georgia Tech, Atlanta, GA, USA.
11. Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA, USA.
12. Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN, USA.
13. Division of Environmental and Ecological Engineering, Purdue University, West Lafayette, IN, USA.
14. Department of Civil Engineering, Purdue University, West Lafayette, IN, USA.
15. Wadsworth Department of Civil and Environmental Engineering, West Virginia University, Morgantown, WV, USA.
16. Department of Civil and Environmental Engineering, Northeastern University, Boston, MA, USA.
17. Department of Environmental Microbiology, Swiss Federal Institute of Aquatic Science and Technology (EAWAG), Dubendorf, Switzerland.

* Corresponding authors
** Co-first authors

Corresponding Authors
William J. Rhoads − Department of Environmental Microbiology, Swiss Federal Institute of Aquatic Science and Technology (EAWAG), Dubendorf, Switzerland; Email: rhoadswj@bv.com.
Current affiliation: Black and Veatch, Kansas City, MO 64114, USA

Emily Garner − Wadsworth Department of Civil and Environmental Engineering, West Virginia University, Morgantown, WV, 26506, USA; Email: emily.garner@mail.wvu.edu.

Kelsey J. Pieper − Department of Civil and Environmental Engineering, Northeastern University, Boston, MA, 02115, USA; Email: k.pieper@northeastern.edu.

Sections

List of Supplemental Figures

List of Supplemental Tables

List of Supplemental Equations

List of Supplemental Text

1. Supplemental Figures

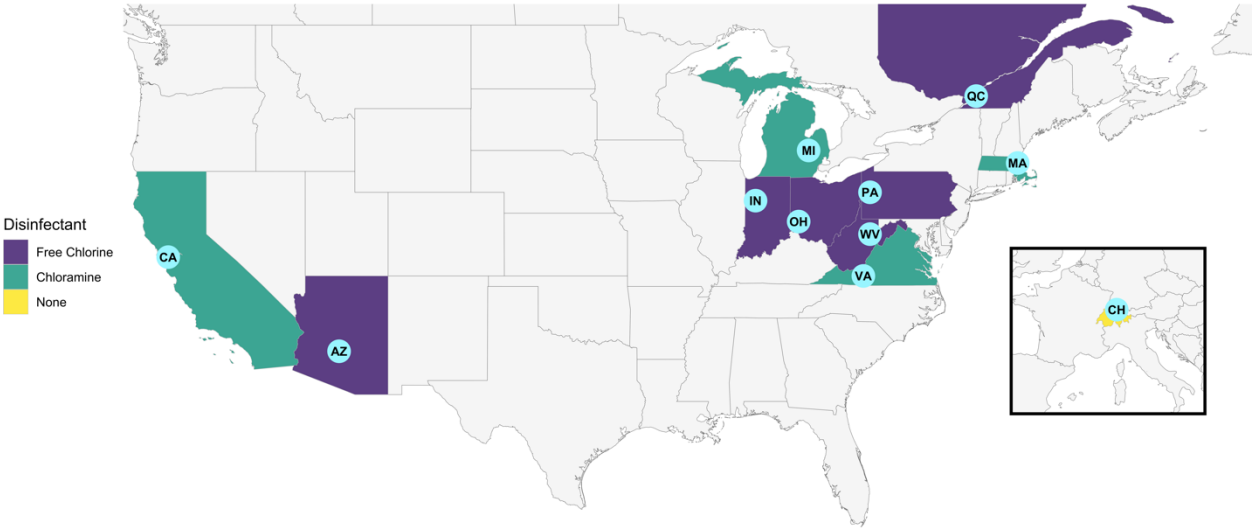

Figure S1. Sampling site geographic locations in the U.S., Canada, and Switzerland as well as distribution system disinfectant type. Sampling site fill color represents the type of secondary disinfectant (free chlorine, chloramine, or no residual disinfectant) used in the distribution system serving the buildings sampled. Number of samples per site: AZ: 7, CA: 20, CH: 62, IN: 12, MA: 12, MI: 19, OH: 4, PA: 18, QC: 56, VA: 18, WV: 30.

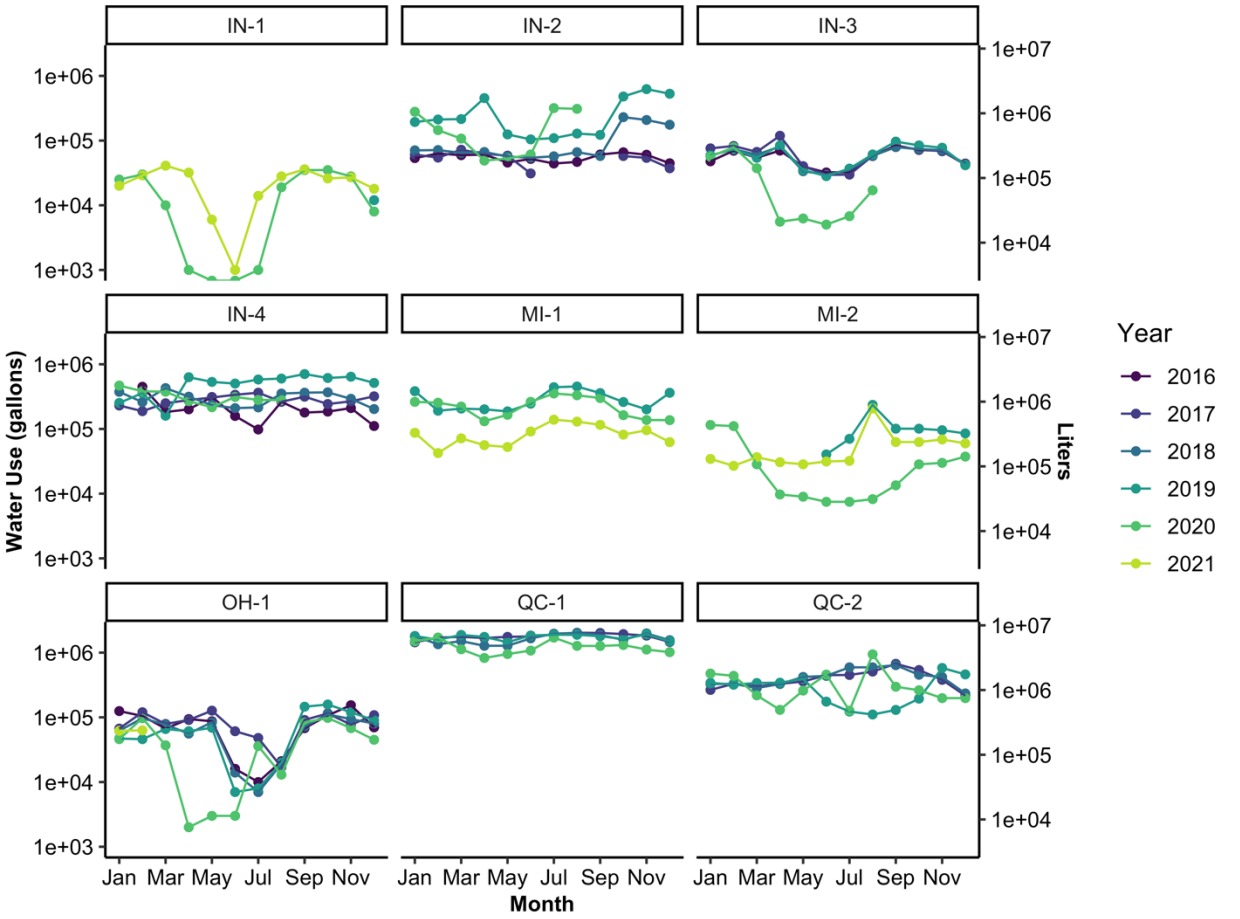

Figure S2. Total monthly building water use for the nine study buildings with available data. IN: Indiana; MI: Michigan; OH: Ohio; QC: Quebec.

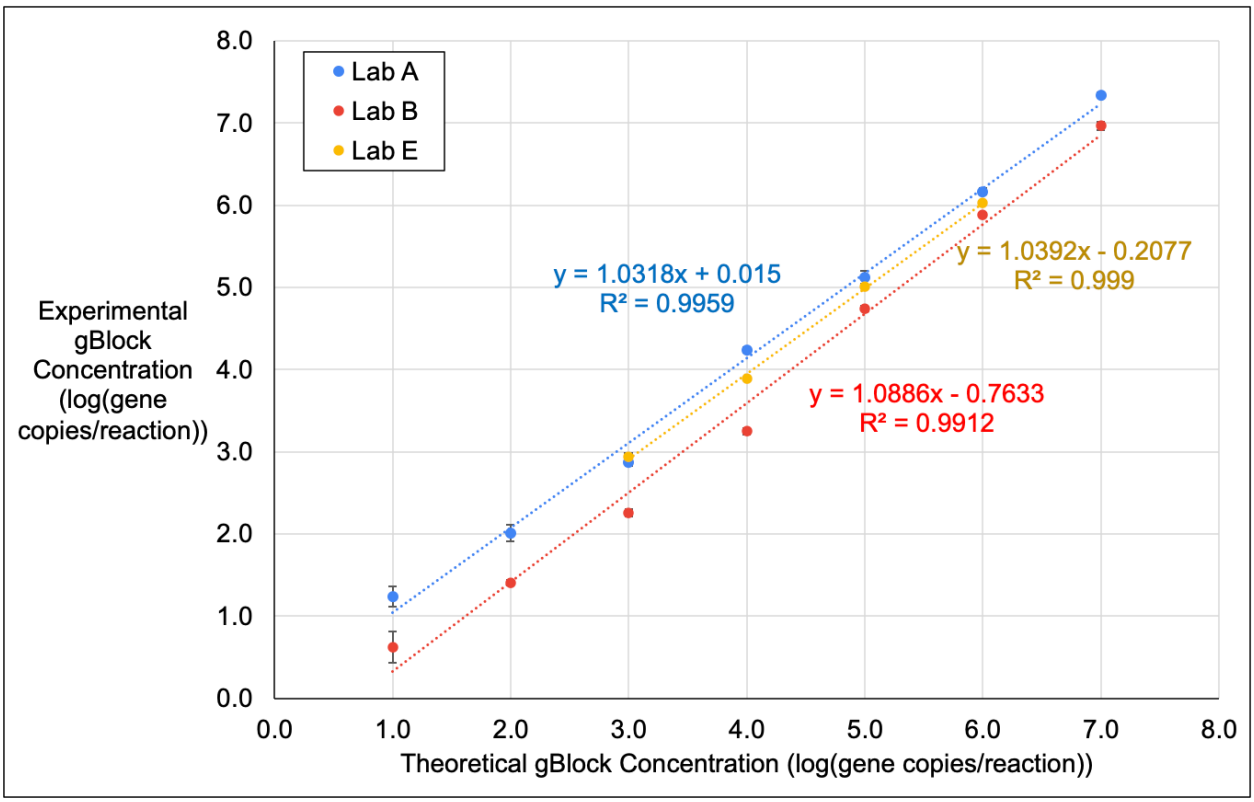

Figure S3. Cross-laboratory validation results for the laboratories using the Nazarian et al. (2008) quantitative polymerase chain reaction (qPCR) assay. Cross-laboratory validation was conducted at Laboratories A, B, and E using a synthetic DNA standard, which consisted of the Nazarian et al. amplicon (79 base pair [bp] with 30 bp neutral adaptors on both ends) at 10^9 copies per microliter (µL, Integrated DNA Technologies [IDT], Coralville, IA, USA). The gBlock was ordered from IDT pre-eluted in nuclease-free water. Upon receipt, the gBlock was divided into 20 µL aliquots, which were frozen at -80°C, then shipped overnight on ice to participating labs. Each laboratory analyzed serial dilutions of the standard to 10^3 gc/µL or 10^1 gc/µL on the same plate as the laboratory's typical standard material. The universally quantified standards were all quantified within the tolerance range such that concentrations can be compared across laboratories.

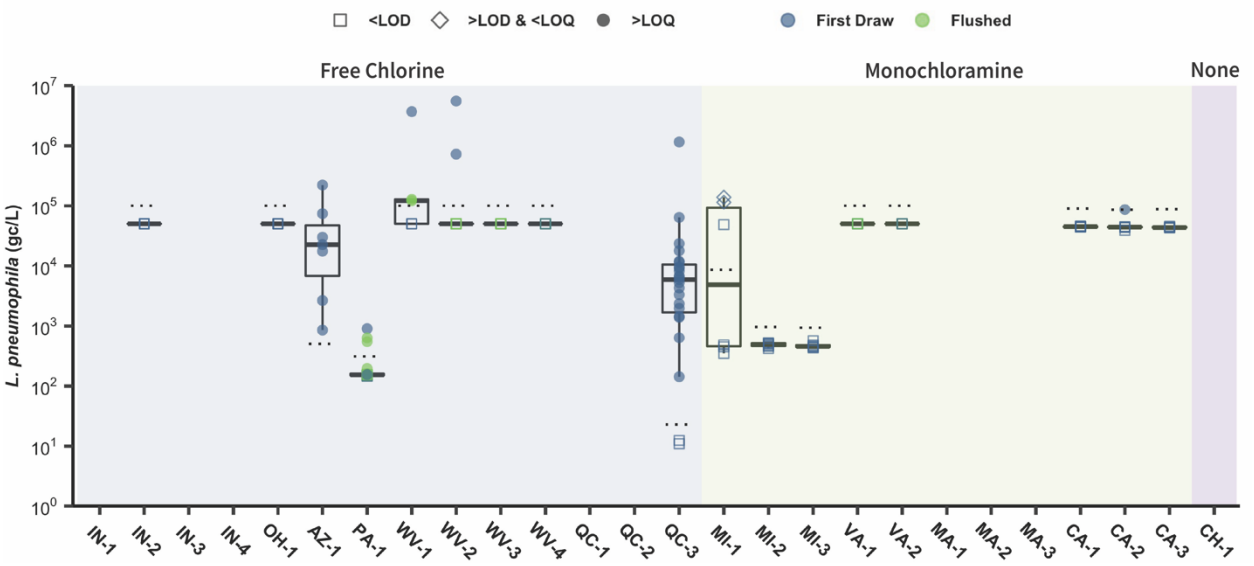

Figure S4. *L. pneumophila* quantitative polymerase chain reaction (qPCR) and droplet digital PCR (ddPCR) results by building and secondary disinfectant type. Marker color represents sample type, where blue circles are first draw samples and green circles are flushed samples. Results below the limit of detection (LOD) are plotted at one-half the LOD and shown as open squares. Results above the LOD but below the limit of quantification (LOQ) are shown as open diamonds. Results above the LOQ are plotted as filled circles. LOD and LOQ thresholds vary by laboratory depending on qPCR/ddPCR sensitivity (Table S9) and concentration/extraction volumes (Table S5). Dotted horizontal lines show the geometric mean of the LOD for each building.

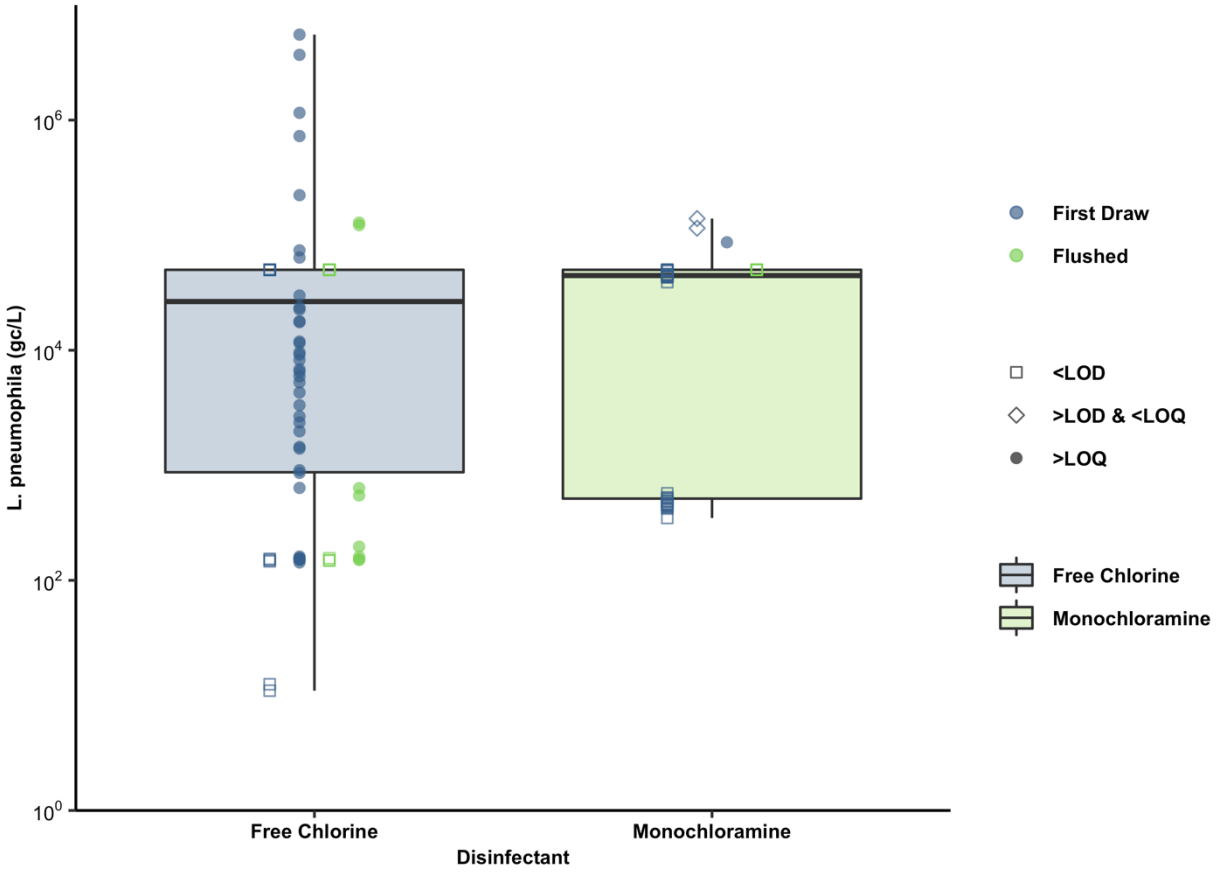

Figure S5. *L. pneumophila* quantitative polymerase chain reaction (qPCR) and droplet digital PCR (ddPCR) results by disinfectant type and sample type. Marker color represents sample type, where blue circles are first-draw samples and green circles are flushed samples. Results below the limit of detection (LOD) are plotted at one-half the LOD and shown as open squares. Results below the above LOD but below the limit of quantification (LOQ) are shown as open diamonds. Results above the LOQ are plotted as filled circles.

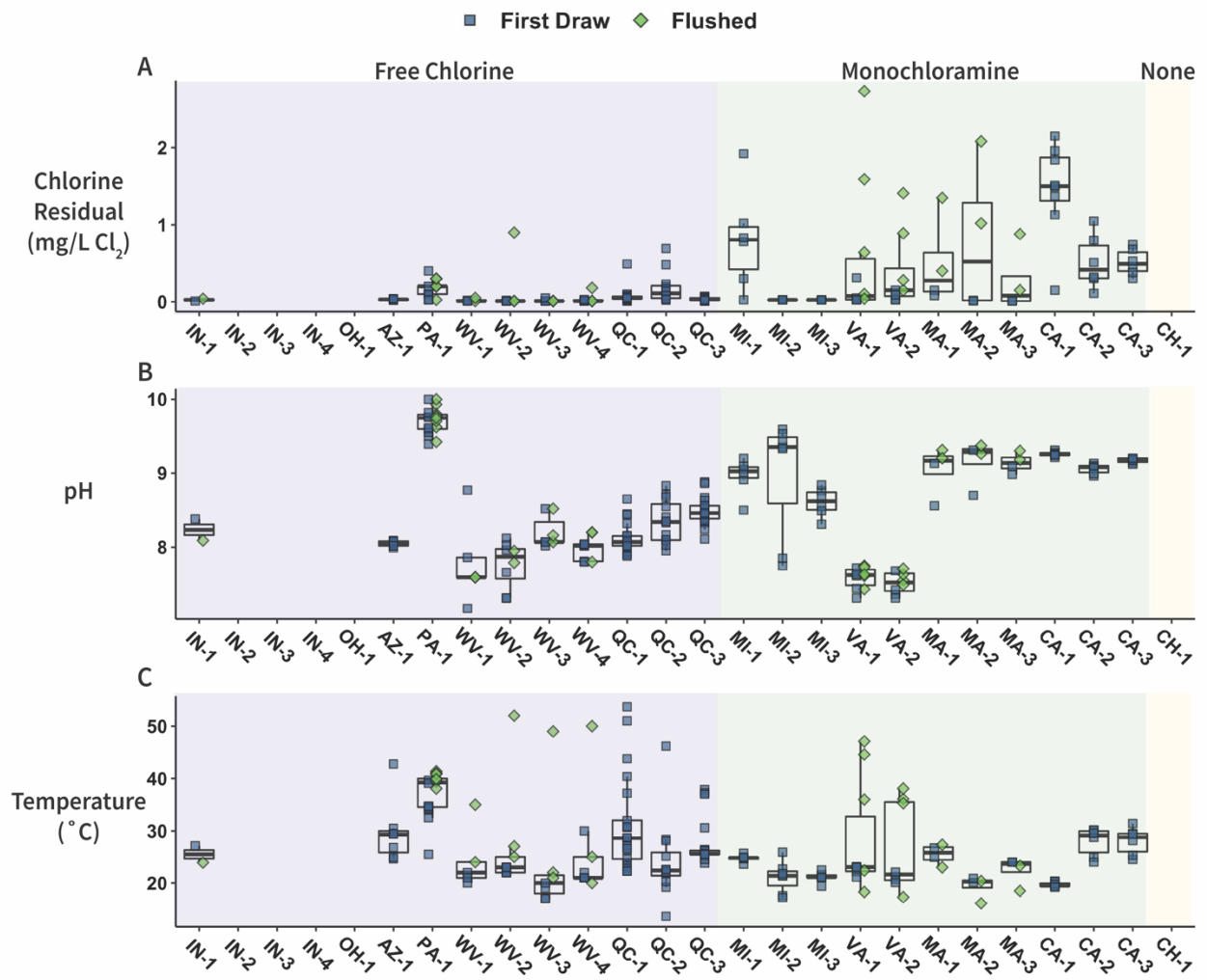

Figure S6. Chlorine residual (A), pH (B), and temperature (C) by building and sample type. Marker color represents sample type, where blue squares are first-draw samples and green diamonds are flushed samples. Background colors represent disinfectant type: free chlorine, chloramine, or none. Temperature and chlorine were not measured for samples collected from the Swiss site (Site CH). IN: Indiana; OH: Ohio; AZ: Arizona; PA: Pennsylvania; WV: West Virginia; QC: Quebec; MI: Michigan; VA: Virginia; MA: Massachusetts; CA: California; CH: Switzerland.

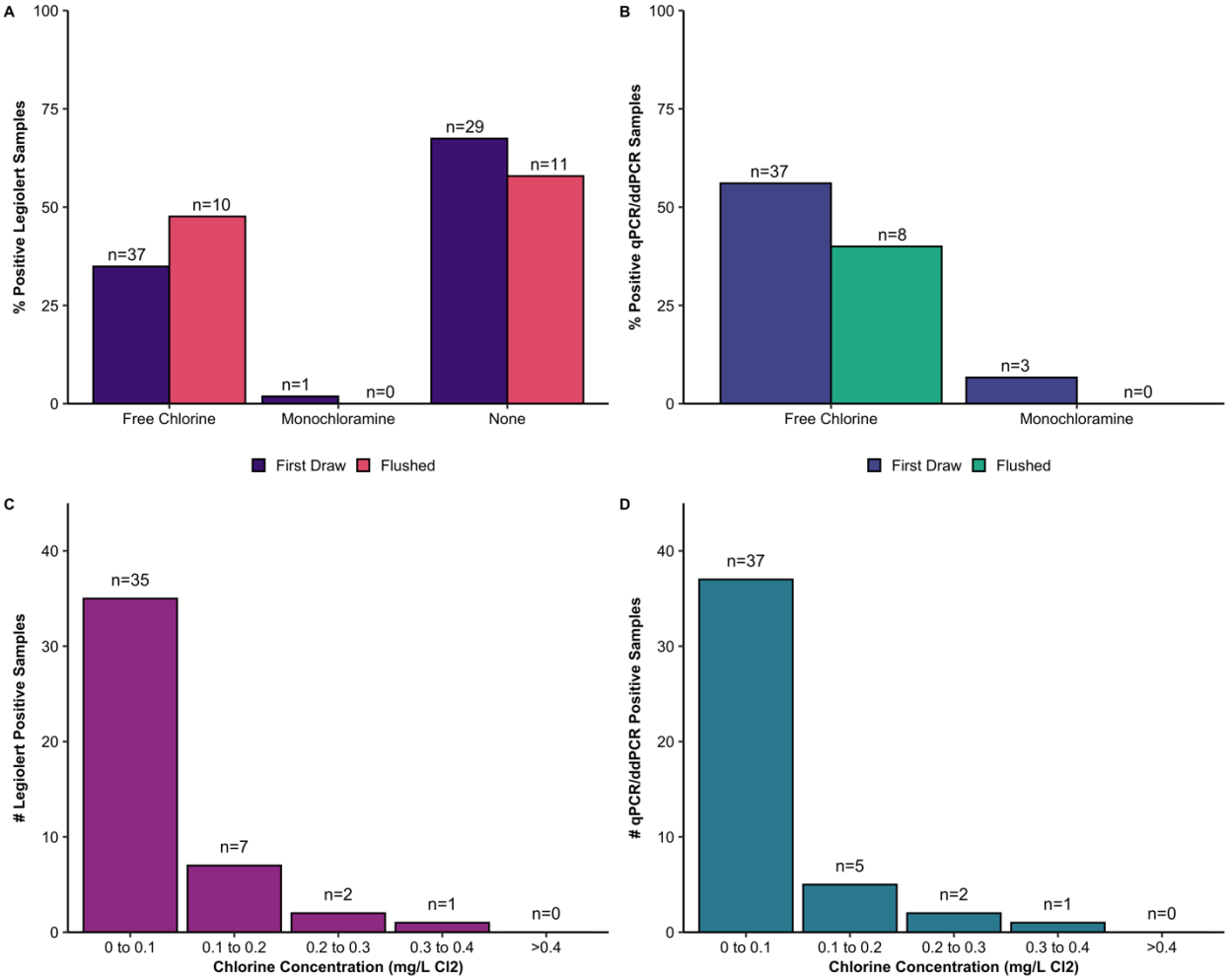

Figure S7. *L. pneumophila* Legiolert and quantitative polymerase chain reaction (qPCR) and droplet digital polymerase chain reaction (ddPCR) results by disinfectant type and concentration and sample type. A) Percent Legiolert-positive samples by disinfectant type and flush condition, B) percent positive qPCR/ddPCR samples by disinfectant type and flush condition, C) number of Legiolert-positive samples by chlorine concentration **for free chlorine first draw and flushed samples only**, D) number of qPCR/ddPCR-positive samples by chlorine concentration **for free chlorine first draw and flushed samples only**.

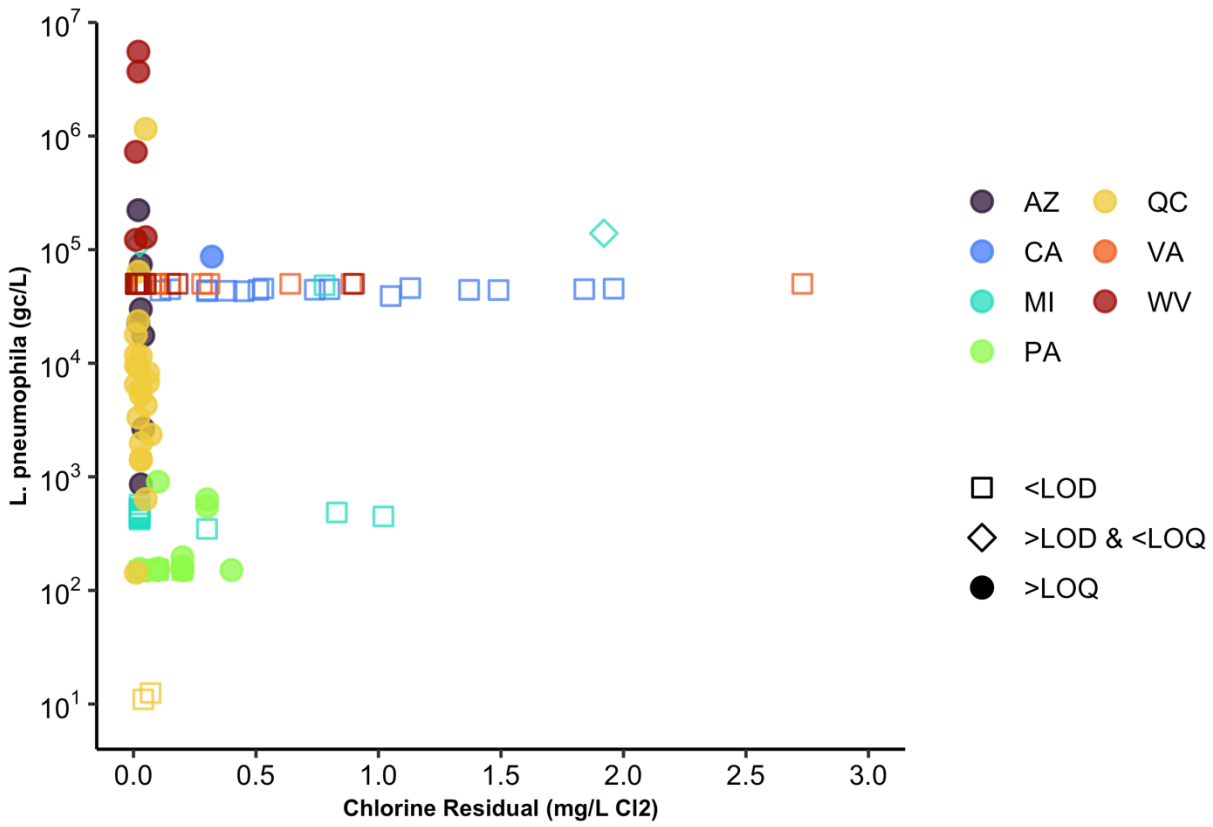

Figure S8. *L. pneumophila* quantitative polymerase chain reaction (qPCR) and droplet digital polymerase chain reaction (ddPCR) results as a function of sample chlorine residual. Results below the limit of detection (LOD) are plotted at one-half the LOD and shown as open squares. Results below the above LOD but below the limit of quantification (LOQ) are shown as open diamonds. Results above the LOQ are plotted as filled circles.

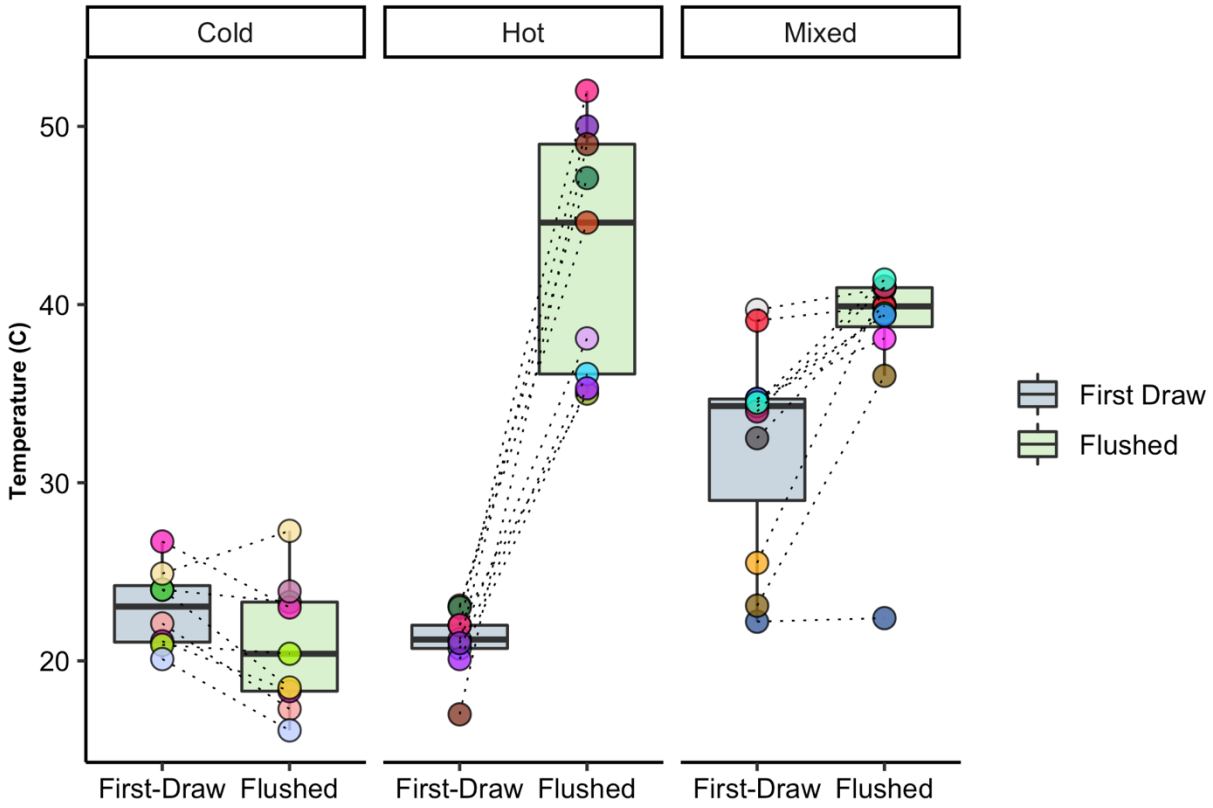

Figure S9. Sample temperature results by condition (first-draw vs. flushed) and fixture temperature (cold, hot, or mixed) for paired samples. Each point is a sample, and point color represents fixture identity.

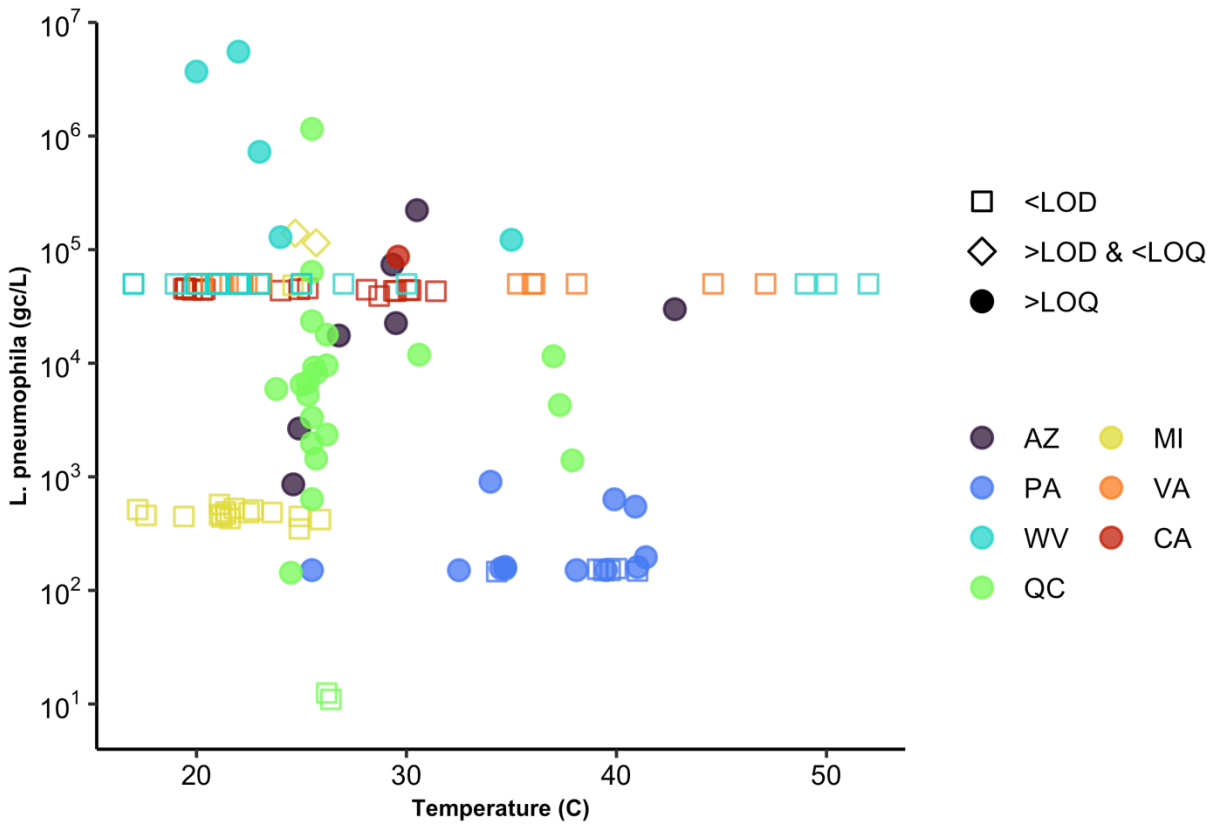

Figure S10. *L. pneumophila* quantitative polymerase chain reaction (qPCR) and droplet digital polymerase chain reaction (ddPCR) results as a function of sample temperature. Results below the limit of detection (LOD) are plotted at one-half the LOD and shown as open squares. Results above the LOD but below the limit of quantification (LOQ) are shown as open diamonds. Results above the LOQ are plotted as filled circles. AZ: Arizona; MI: Michigan; PA: Pennsylvania; VA: Virginia; WV: West Virginia; CA: California; QC: Quebec.

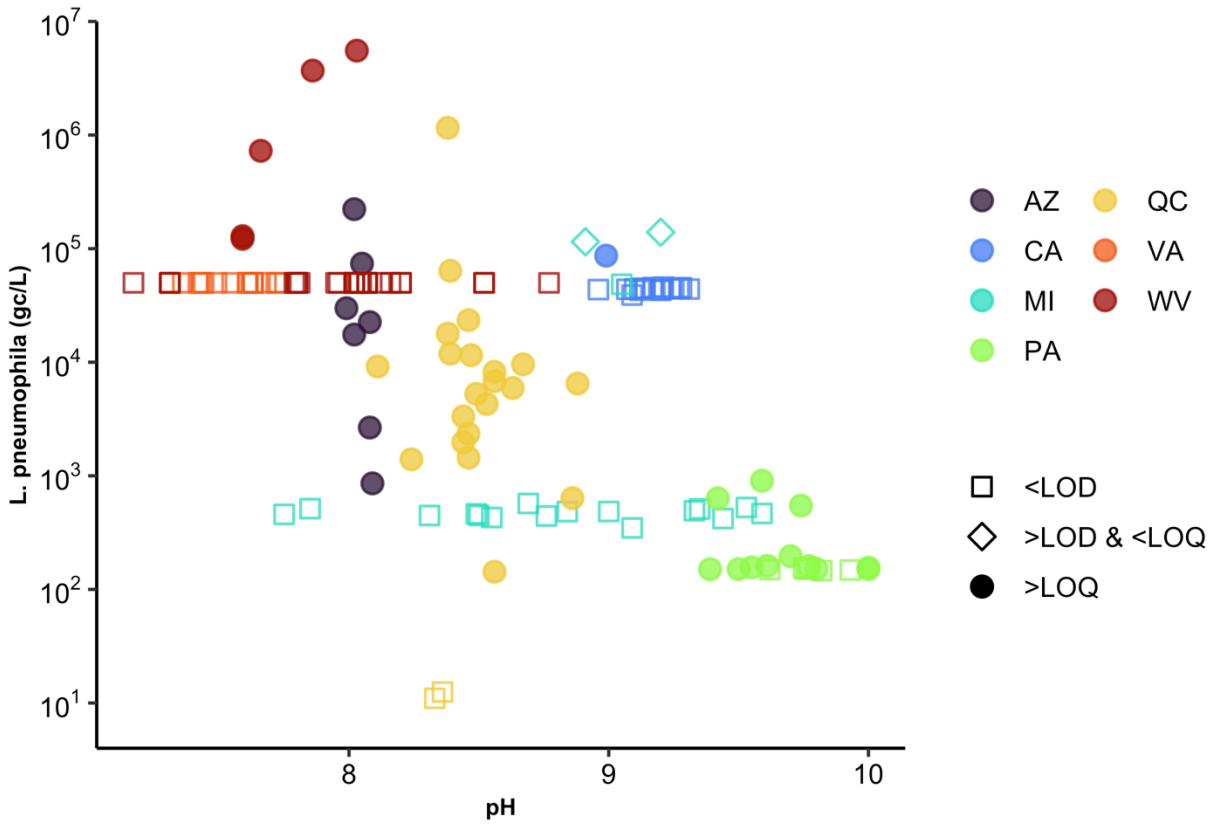

Figure S11. *L. pneumophila* quantitative polymerase chain reaction (qPCR) and droplet digital polymerase chain reaction (ddPCR) results as a function of sample pH. Results below the limit of detection (LOD) are plotted at one-half the LOD and shown as open squares. Results above LOD but below the limit of quantification (LOQ) are shown as open diamonds. Results above the LOQ are plotted as filled circles. AZ: Arizona; QC: Quebec; CA: California; VA: Virginia; MI: Michigan; WV: West Virginia; PA: Pennsylvania.

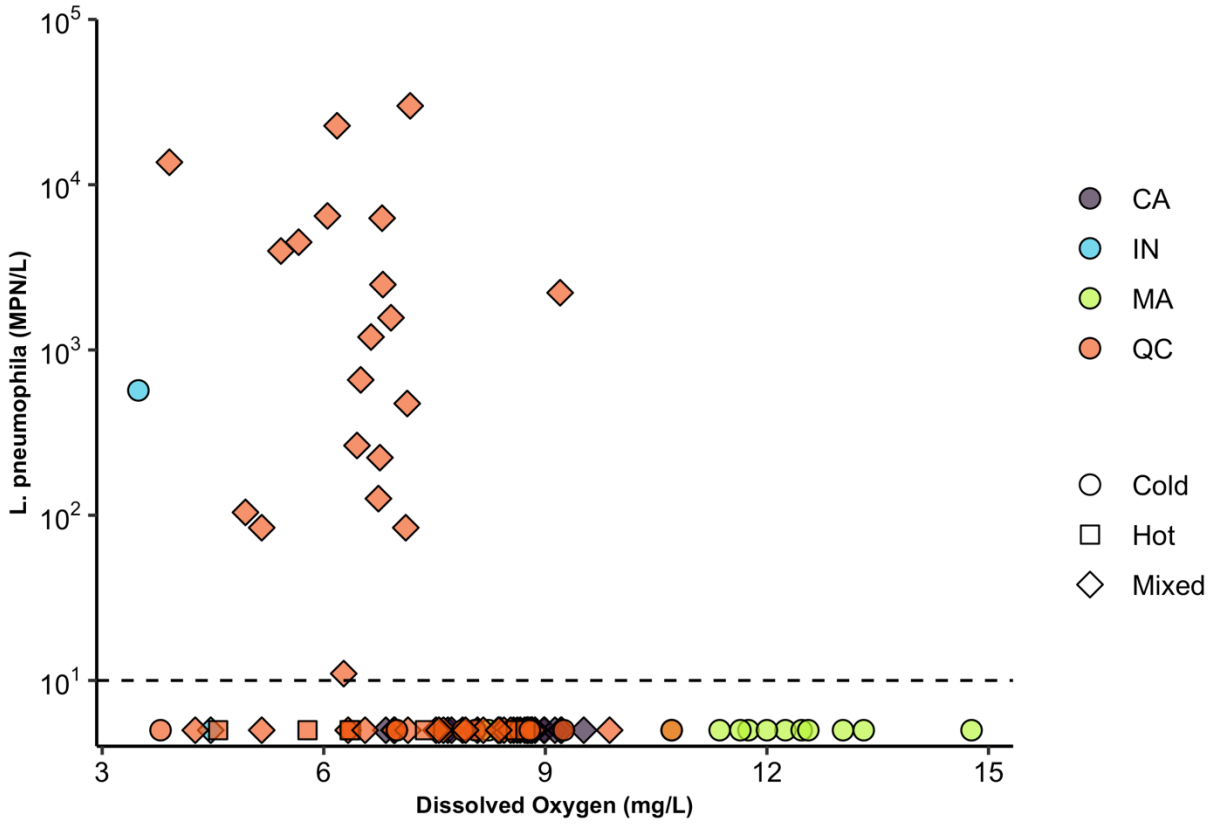

Figure S12. Dissolved oxygen as a function of culturable *L. pneumophila.* MPN/L: Most probable number/L. CA: California; IN: Indiana; MA: Massachusetts; QC: Quebec.

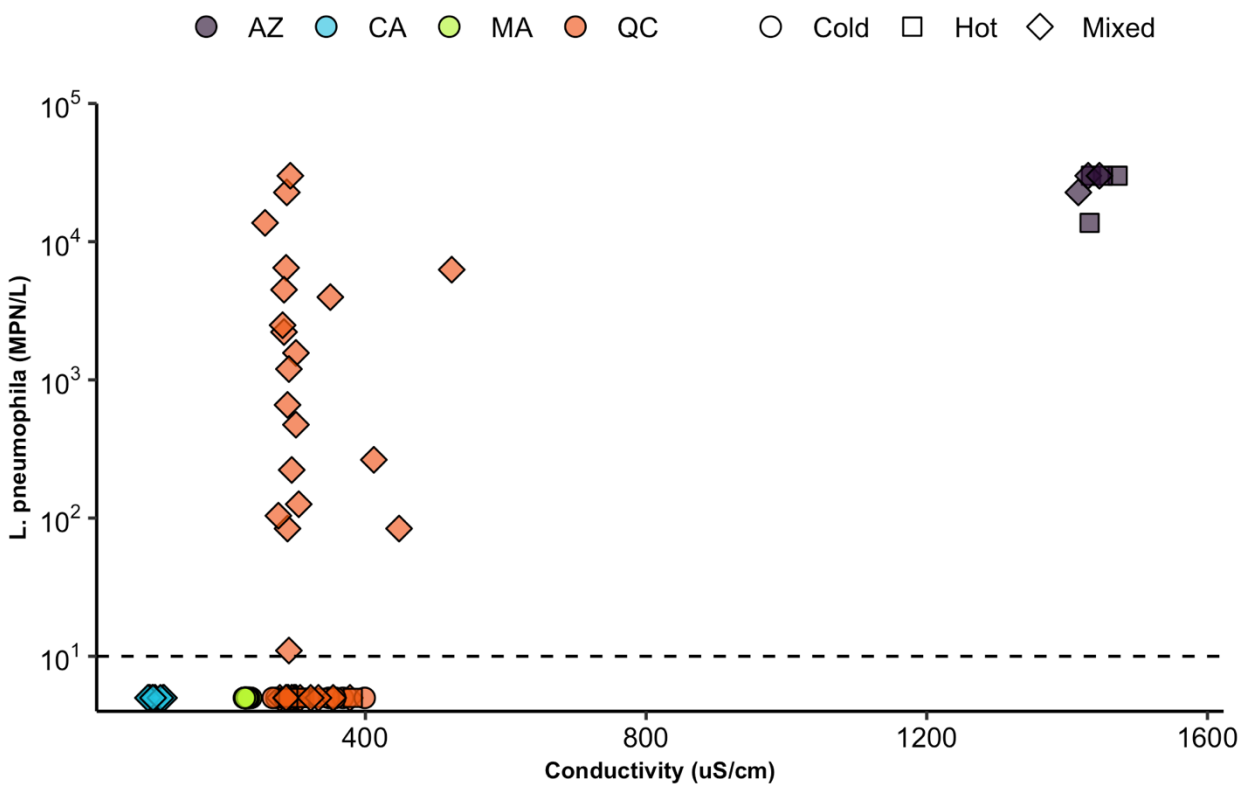

Figure S13. Electrical conductivity as a function of culturable *L. pneumophila.* MPN/L: Most probable number/L. AZ: Arizona; CA: California; MA: Massachusetts; QC: Quebec.

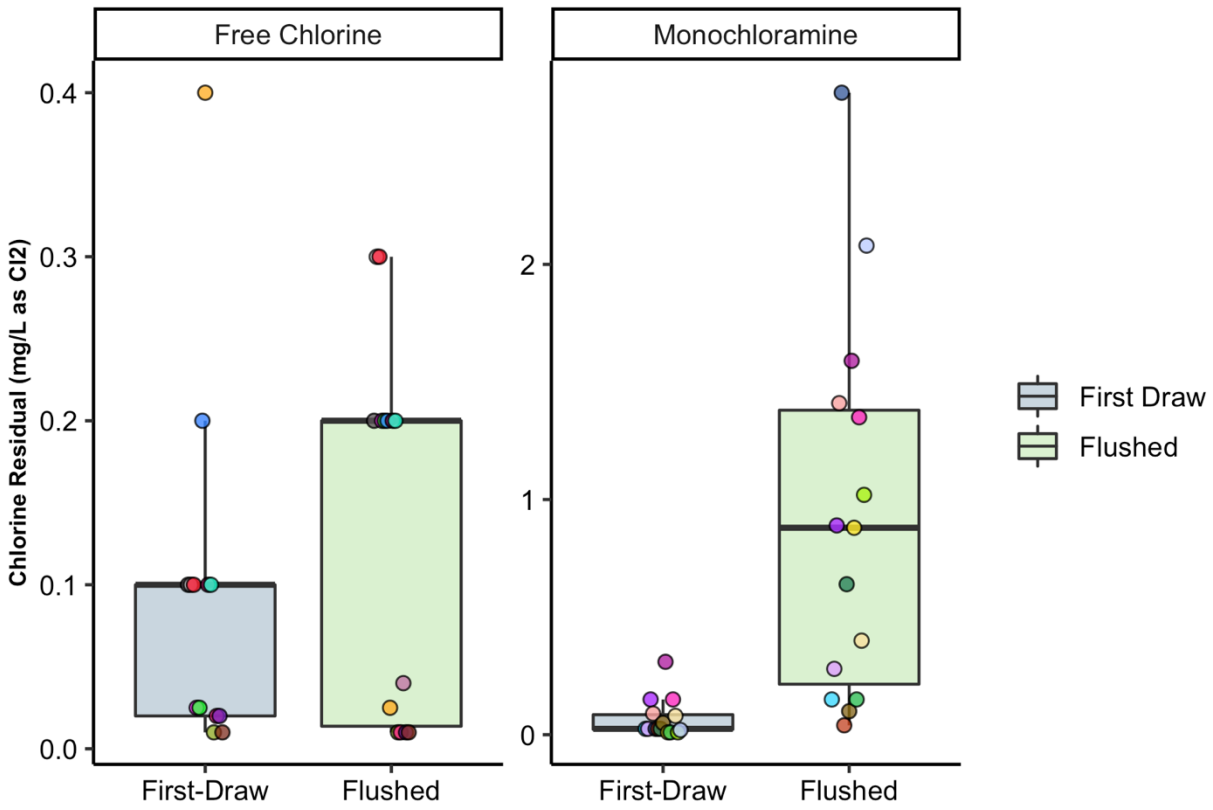

Figure S14. Sample chlorine residual results by condition (first draw vs. flushed) and disinfectant type (free chlorine and chloramine) for paired samples. Points are colored by fixture.

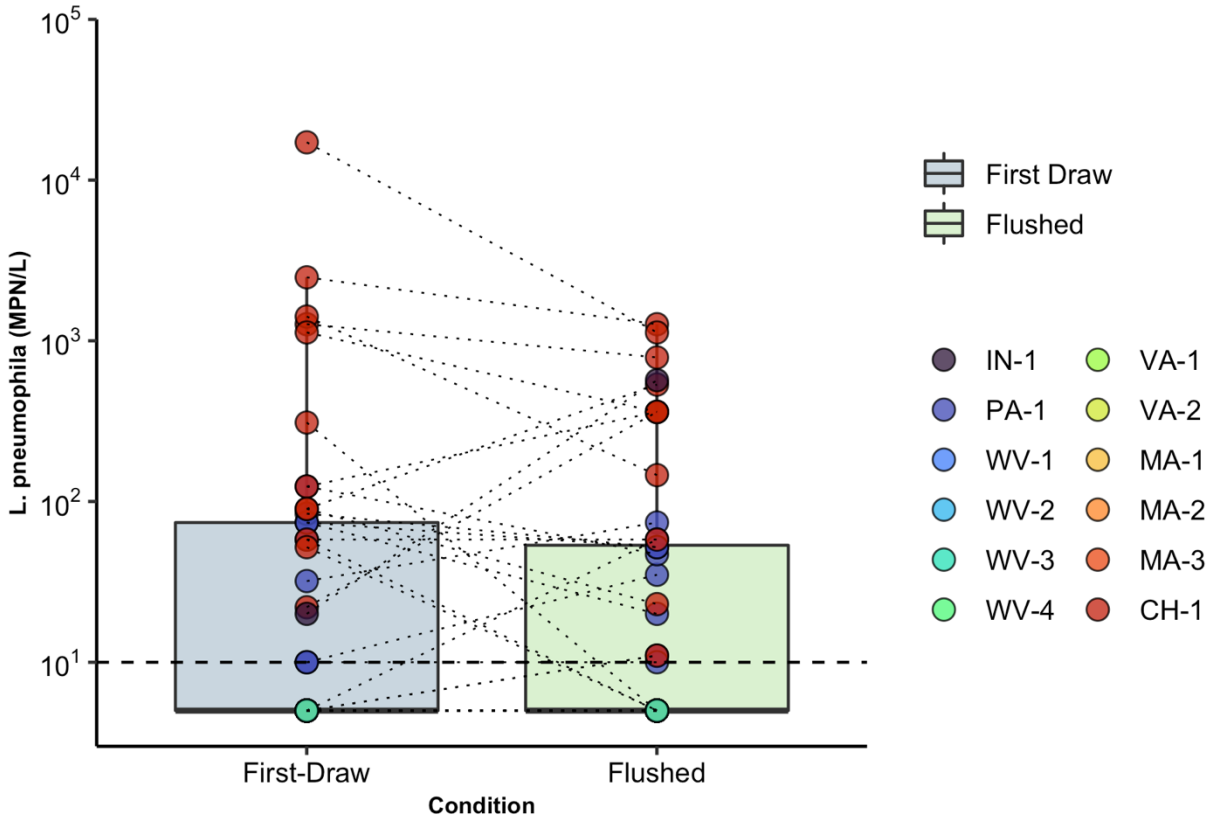

Figure S15. *L. pneumophila* Legiolert results (MPN/L) by condition (first draw vs. flushed) for paired samples. MPN/L: Most probable number/L. Points are colored by building. The dashed line represents the limit of detection (LOD). IN: Indiana; VA: Virginia; PA: Pennsylvania; WV: West Virginia; MA: Massachusetts; CH: Switzerland.

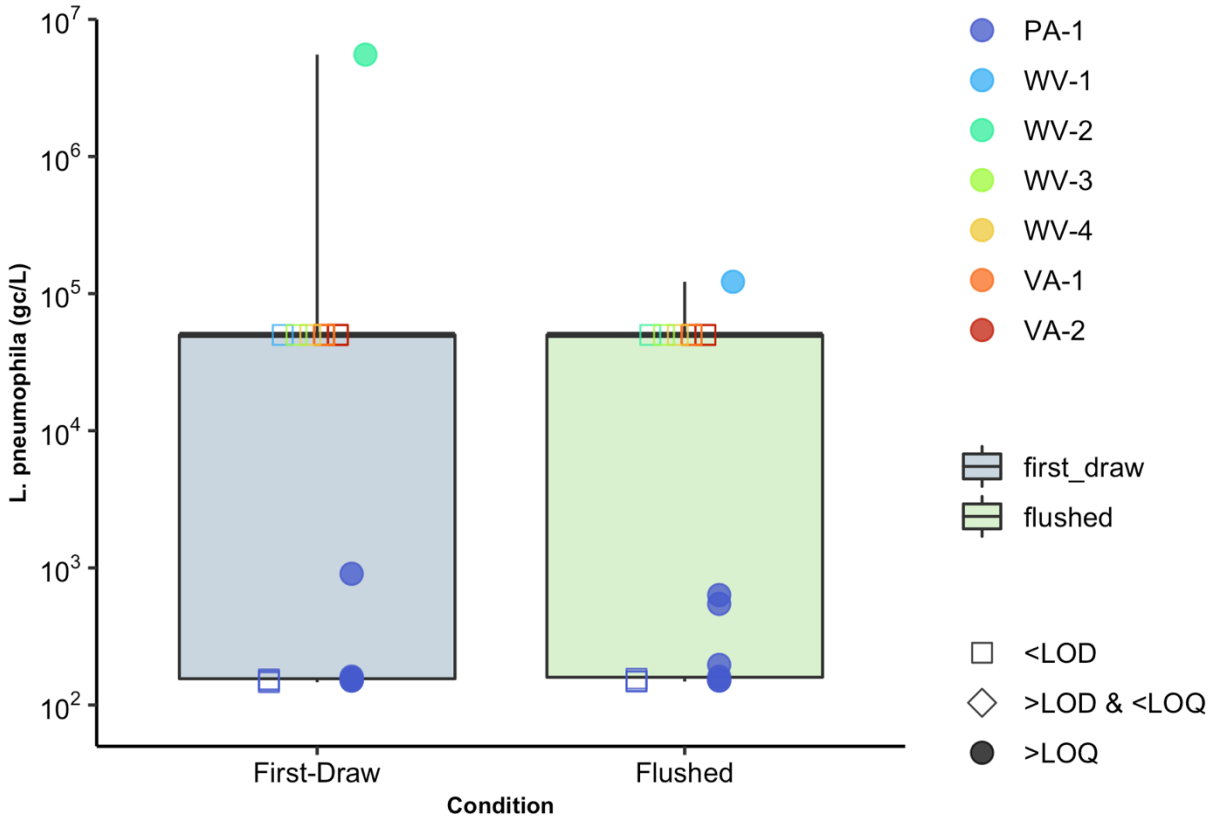

Figure S16. *L. pneumophila* quantitative polymerase chain reaction (qPCR) and droplet digital polymerase chain reaction (ddPCR) results (gc/L) by site for paired samples. Results below the limit of detection (LOD) are plotted at one-half the LOD and shown as open squares. Results above the LOD but below the limit of quantification (LOQ) are shown as open diamonds. Results above the LOQ are plotted as filled circles. Points are colored by building. PA: Pennsylvania; WV: West Virginia; VA: Virginia.

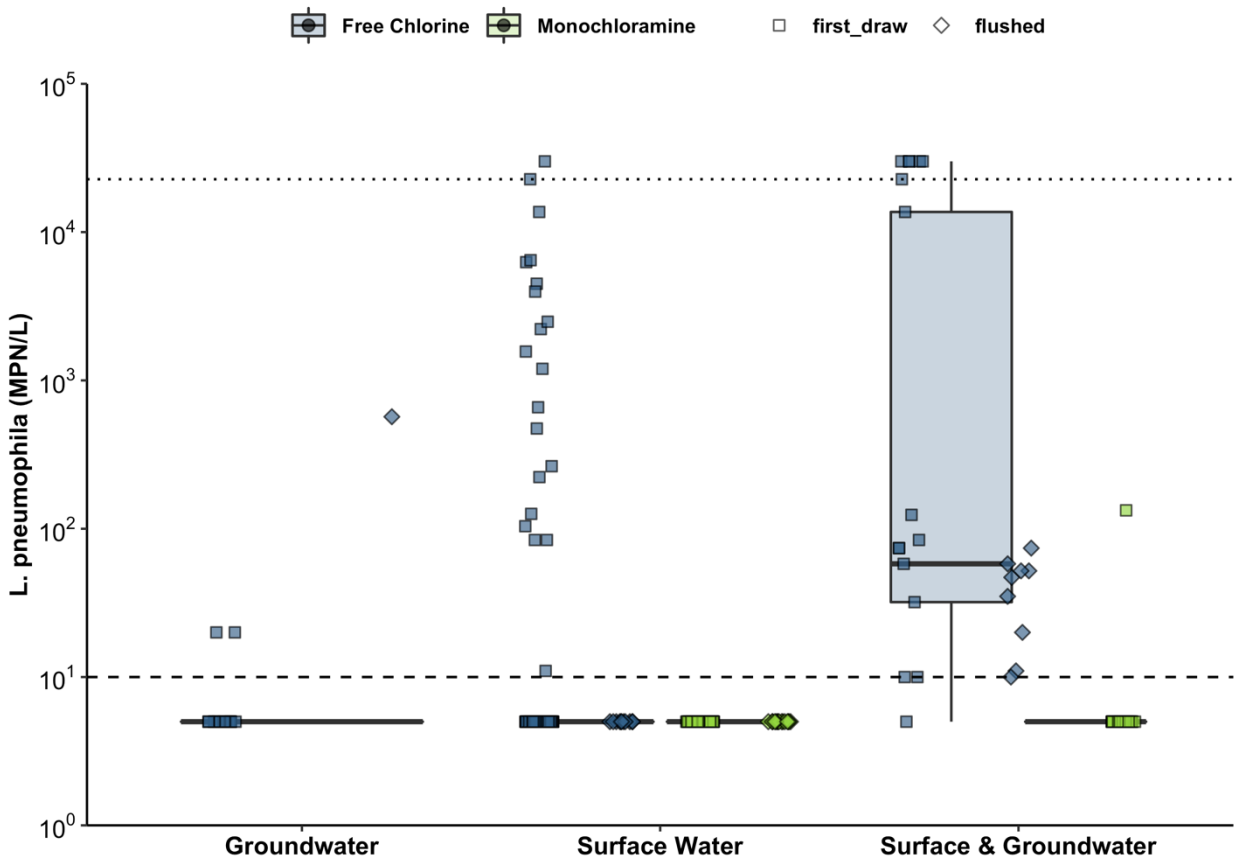

Figure S17. *L. pneumophila* Legiolert results (MPN/L) by source water type, disinfectant type, and sample type. MPN/L: Most probable number/L. Marker and bar color represents disinfectant type, with blue being free chlorine and green being monochloramine. Marker shape represents flush condition, where squares are first-draw samples and diamonds are flushed samples. The dashed line represents the limit of detection (LOD; 10 MPN/L) and the dotted line represents the upper limit of quantification (ULOQ, >22,726 MPN/L). Results below the LOD are plotted at one-half the LOD (5 MPN/L). Results above the ULOQ are plotted as 30,000 MPN/L.

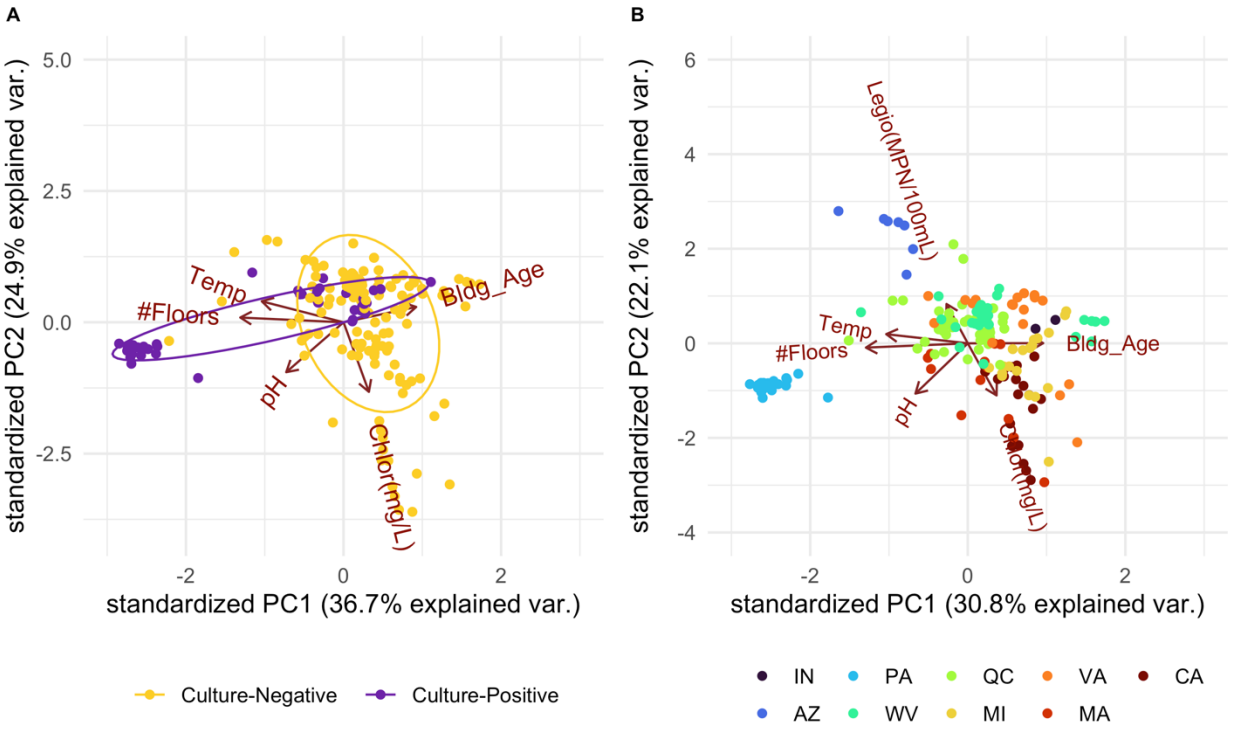

Figure S18. Principal component analysis (PCA) results, incorporating physicochemical parameters (chlorine residual, temperature, and pH) and building characteristics (number of floors and building age). A) Samples colored by culture-positivity or -negativity. B) Samples colored by site and axes include Legiolert concentration of *L. pneumophila*. IN: Indiana; PA: Pennsylvania; QC: Quebec; VA: Virginia; CA: California; AZ: Arizona; WV: West Virginia; MI: Michigan; MA: Massachusetts.

Chlorine concentrations explained much of the variance in negative samples (A). Site PA clustered separately from the other sites, possibly because it was a separate replicated experimental system. Most of the variance in Site AZ was explained by *L. pneumophila* culture concentrations; whereas variance in other sites were mostly explained by other factors (B).

2. Supplemental Tables

Table S1. Additional buildings, sampling, occupancy, and preventative measure information.

Site	Building	State or Region	Disinfect.	Type	Hot Water Recirc.	Closure Date	Sampling Date(s)	Description of Occupancy/Closures	Preventative Measures During Low Occupancy Period	Measures after Study, Follow-Up, or Response Actions
IN	IN-1	Indiana	Free Chlorine	Full-Scale	Yes	3/23/20	8/5/20, 8/12/20	Completely unoccupied.	No preventative measures were taken prior to sampling.	Flushing was performed after *L. pneumophila* was detected by Legiolert. Follow-up sampling was then conducted.
	IN-2				Yes	3/23/20	7/21/20	Mostly unoccupied.		Some flushing was conducted by the building operators.
	IN-3				Yes	3/23/20	7/23/20			
	IN-4				Yes	3/23/20	7/23/20			
OH	OH-1	Ohio	Free Chlorine	Full-Scale	Yes	3/15/20	8/15/20	Building was closed from March - September 2020	The utility operator flushed the building in early August to attempt to get a consistent chlorine residual.	Flushing and shock chlorination performed after *L. pneumophila* detection.
AZ	AZ-1	Arizona	Free Chlorine	Full-Scale	Yes	3/15/20	8/24/20	At the lowest occupancy during COVID-19 pandemic (March 2020), occupancy was reduced to 15-25% of normal occupancy. By Aug-Sept 2020 during the sampling period, occupancy rose to 30-50% and 40-70% respectively, depending on the floor considered (1-5).	No preventative measures prior to *L. pneumophila* detection by this study. Some previous studies on physical chemical water quality indicated other issues in the building such as lack of chlorine residual (potential removal by water softener) and DBP formation, however no actions taken until to *L. pneumophila* detection.	Building was flushed after to *L. pneumophila* detection. Shower was flushed for 5 h by the facilities manager. 9/4/20 sinks, janitor's closets, and showers were flushed for 30 mins on every floor in stages of even/odd floors. Water heater set point was turned to 140F and allowed to recirculate 30-35 mins then returned to 115 degrees. 9/8/20 janitors did periodic (unspecified) flushing. Resin tanks regenerated on a weekly basis.
PA	PA-1	Pennsylvania	Free Chlorine	Lab-Scale	No	3/19/20	7/22/20	Shower rig was completely stagnant prior to sampling. Building was significantly stagnant until early June when partial re-opening began.	No preventative measures were taken prior to sampling.	None
WV	WV-1	West Virginia	Free Chlorine	Full-Scale	Yes	3/13/20	8/7/20	Occupancy reduced to ~5% as of 3/13/20	No preventative measures were taken prior to sampling.	None
	WV-2				Yes	3/13/20	8/7/20	Occupancy reduced to ~2% as of 3/13/20		
	WV-3				Yes	3/13/20	8/7/20	Unoccupied except for occasional maintenance.		
	WV-4				Yes	3/13/20	8/7/20			
QC	QC-1	Quebec (CA)	Free Chlorine	Full-Scale	Yes	3/13/20	5/14/20	Occupancy reduced to approximately 2% as of 3/13/20, increased to approximately 5% over the summer.	Building water was being used by the HVAC system but was not intentionally flushed or managed.	Full recommissioning flushing following Quebec's procedures performed on 5/8/20. Building engineers designed a flushing plan for all water points. Building has a newly developed flushing plan.
	QC-2				Yes	3/13/20	5/5/2020			
	QC-3				Yes	3/13/20	12/7/20	Approx. < 5%; no visitors as of 3/13/20 (only maintenance and managers). Day camps as of July 1st, 2020 (no shower use, but increased occupancy)	Building water was being used by the HVAC system but was not intentionally flushed or managed. Partial recommissioning flushing (only showers were flushed for 5-min, mitigated water) on 7/14/20. Showers remained closed until now due to elevated *L. pneumophila* concentrations.	Building was flushed (only showers, mitigated water, 5-min) on 7/14/20. Showers remained close until 2021.

Site	Building	State or Region	Disinfect.	Type	Hot Water Recirc.	Closure Date	Sampling Date(s)	Description of Occupancy/Closures	Preventative Measures During Low Occupancy Period	Measures after Study, Follow-Up, or Response Actions
MI	MI-1	Michigan	Mono-chloramine	Full-Scale	Yes	3/14/20	8/21/20	Occupancy was restricted to essential personnel (~25%).	Closed water fountains. Random fixture flushing. Pool was being refilled weekly to flush water.	Newly developed flushing and building recommissioning guidelines.
	MI-2				Yes	3/14/20	8/24/20		Closed water fountains. Cold water in the building was being flushed every two weeks. Building was last flushed prior to sampling on 8/4/20.	
	MI-3				Yes	3/14/20	8/26/20		Building water was being sparsely used but not intentionally flushed or managed.	Resampled due to *L. pneumophila* detection. Reported water heater setpoint was 120F (49C), despite lower temps even after extended flushing. After detection, hot water tanks were drained. Full building flush conducted for 15 minutes on 9/5/20. Newly developed flushing and building recommissioning guidelines.
VA	VA-1	Virginia	Mono-chloramine	Full-Scale	Yes	3/16/20	7/26/20	Occupancy was restricted to essential personnel.	One-time flushing event April (week of 4/20/2020). Opened most outlets for 1-3 minutes.	None
	VA-2				Yes	3/16/20	7/28/20			
MA	MA-1	Massachusetts	Mono-chloramine	Full-Scale	Yes	3/13/20	6/5/20	Occupancy reduced to ~5% as of 3/23/20, phased reopening, starting June 2020	From the water usage data, the building water was sparsely used. Water usage increased by 4 logs with phased reopening, starting June 2020.	Maintenance activities (cleaning) likely in the building during the stagnation period, so minor water usage was likely.
	MA-2				Yes	3/13/20	6/5/20			
	MA-3				Yes	3/13/20	6/5/20			
CA	CA-1	California	Mono-chloramine	Full-Scale	Yes	4/1/20	7/16/20	Occupancy reduced to 2-4% in April, then 0% in June 2020	No preventative measures were taken during the study period.	No preventative action within the buildings. Maintenance activities (WIFI repairs and cleaning) meant there may have been minor water usage.
	CA-2				Yes	4/1/20	7/16/20			
	CA-3				Yes	4/1/20	7/16/20			
CH	CH-1	Switzerland	None	Full-Scale	Yes	3/9/20	4/24/20	5% occupancy from 3/9/20 to April 28, 2020; maximum 30% occupancy 4/28/20 through the end of the year	Swiss Federal Guidelines: Flush all fixtures until maximum temperature is reached. Did this one time before reopening the building. Emptied the boiler hot water during flushing several times.	None

Table S2. Summary of total number of samples totals by site, flush condition, and type of analysis.

	Free Chlorine Systems						Chloramine Systems				No Residual System	
Category	**IN**	**OH**	**AZ**	**PA**	**WV**	**QC**	**MI**	**VA**	**MA**	**CA**	**CH**	**Total**
Total Samples	12	4	7	18	30	56	19	18	12	20	62	**258**
First Draw	11	4	7	9	19	56	19	9	6	20	43	**203**
Flushed	1	--	--	9	11	--	--	9	6	--	19	**55**
Legiolert	12	4	7	18	30	56	19	18	12	20	62	**258**
qPCR	4	4	7	--	30	23	19	16	--	17	--	**120**
ddPCR	--	--	--	18	--	--	--	--	--	--	--	**18**
Chlorine (Total/Free)	2	--	7	18	30	56	19	18	12	20	--	**182**
Temperature	2	--	7	18	30	56	19	18	12	20	--	**182**
pH	2	--	7	18	29	56	19	18	12	20	--	**181**

Table S3. Summary of sampling and analysis controls. A subset of these controls was analyzed by each site.

Control	Description	Analysis
Trip Control	1.1 L of autoclaved Milli-Q/Nanopure water that was kept in the cooler during sampling trips.	Legiolert and qPCR/ddPCR
Environmental Control	1.3 L of autoclaved Milli-Q/Nanopure water that was brought to the site and opened to expose it to the site environment.	Legiolert and qPCR/ddPCR
Legiolert Reagents/Materials Negative Control	100 mL of autoclaved Milli-Q/Nanopure water added to an unused sample container and processed with samples	Legiolert
Legiolert Kit Lot Negative Control	Per manufacturer instructions, *Enterococcus faecalis* ATCC 29212 performed at least once per kit lot	Legiolert
Legiolert Kit Positive Control	Per manufacturer instructions, *L. pneumophila* performed at least once per kit lot	Legiolert
Filtration Set-Up Control	Sterile water filtered using filtration set-up.	Legiolert and qPCR/ddPCR
Filter Control	Unused filter.	qPCR/ddPCR
DNA Extraction Control	Empty tube processed with samples.	qPCR/ddPCR
qPCR/ddPCR Negative Control	Reaction mix with sterile water instead of sample.	qPCR/ddPCR
qPCR/ddPCR Positive Control	Synthetic DNA (gBlock)	qPCR/ddPCR

Table S4. Summary of physicochemical methods.

Site	Free Chlorine		Total Chlorine		Monochloramine		pH	Temperature	Conductivity	Dissolved Oxygen
	Method	DL (mg/L as Cl_2)	Method	DL (mg/L as Cl_2)	Method	DL (mg/L as Cl_2)				
IN & OH	DPD method HACH Pocket Colorimeter DR300	0.02	DPD method HACH Pocket Colorimeter DR300	0.02	NA		Oakton 450 pH probe	Oakton 450 pH probe	NA	YSI ProODO Optical Dissolved Oxygen Instrument
AZ	DR 900 colorimeter - program 80 - DPD 8021 method	0.02	DR 900 colorimeter - program 80 - DPD 8167 method	0.02	NA		Oakton pH30 probe (pH30 pH tester)	Ryobi IR002 Infrared Thermometer	Thermo scientific - Orion Versa Star Pro - pH/ISE/Conductivity/ Dissolved Oxygen Multiparameter Benchtop Meter	
PA	DPD method- Hach Method 8021	0.02	DPD Method - Hach Method 10250	0.05	NA		Hanna Combo Multiprobe		NA	NA
WV	DPD method- Hach Method 8021	0.02	NA		NA		Thermo Scientific Orion Star A326 Portable Meter			NA
QC	DPD method 8021, HACH DR 2800 portable spectrophotometer	0.02	DPD method 8167, HACH DR 2800 portable spectrophotometer	0.02	NA		HACH HQ40d digital portable multi-probes meter	Digital thermometer	HACH HQ40d digital portable multi-probes meter	HACH HQ40d digital portable multi-probes meter
MI	DPD method- Hach Method 10245	0.05	DPD Method - Hach Method 10250	0.05	Indophenol Method- Hach Method 10200	0.04	Hanna Instruments HI98121 portable probe		NA	NA
VA	DPD method- Hach Method 8021	0.02	DPD Method - Hach Method 10250	0.05	NA		Thermo Scientific Orion 110 Series meter with ATC		NA	NA
MA	NA		DPD Method - Hach Method 8167	0.02	NA		Thermo Scientific Orion Star A325 Multiparameter Meter			
CA	DPD method- Hach Method 8021	0.02	DPD Method - Hach Method 8167	0.02	NA		Thermo Scientific Orion STAR A326 Portable Meter			
CH	NA									

DL: Detection limit
NA: Analysis not performed.

Table S5. DNA collection, extraction, and quantification.

Site	Filter Type	Sample Preservation and Storage	DNA Extraction Method	Sampling and Extraction Controls	DNA Quantification
IN & OH	0.4 µm polycarbonate membrane filter disks filters (EMD Millipore, HTTP04700).	Filters aseptically transferred to microcentrifuge tubes and stored at -80˚C until DNA extraction.	DNeasy Power Water Kit (QIAGEN, 14900-100-NF)	An extraction negative control and a filter negative control were included for each extraction session.	NanoDrop
AZ	0.2 µm polycarbonate membrane filter disks (EMD Millipore, GTTP04700)	Filters aseptically transferred to 2 mL microcentrifuge tubes and stored at -80˚C until DNA extraction.	DNeasy Power Soil Pro kit (QIAGEN, 47014). For bead beating - Precellys evolution which was set to 10,000 rpm, 3 cycles for 15 sec with 10 sec pause.	Trip, environmental (field blank), and filter negative controls collected during each sampling event. An extraction negative control was included for each extraction session.	Thermo Scientific NanoDrop 2000 spectrophotometer
PA	0.2 µm polycarbonate membrane filter disks (EMD Millipore, GTTP04700)	Filters aseptically transferred to 2 mL sterile microcentrifuge tubes and stored at -20˚C until extraction	FastDNA SPIN kit (MP Biomedicals, 116540600-CF) with bead beating instead of the FastPrep step.	Trip, environmental, filter, and filtration set-up negative controls collected during each sampling event. An extraction negative control was included for each extraction session.	No DNA quantification
WV	0.2 µm polycarbonate membrane filter disks (EMD Millipore, GTTP04700)	Filters aseptically transferred to 2 mL sterile screw top tubes and stored at -20˚C until extraction	FastDNA SPIN kit (MP Biomedicals, 116540600-CF) with bead beating instead of the FastPrep step.	Environmental and field negative controls included each sampling day. Filter control included in each extraction session.	No DNA quantification
QC	0.2 µm polyethersulfone membrane filter disks (PALL Corporation, 66234)	Filters aseptically transferred to sterile microcentrifuge tubes and stored at -80˚C until DNA extraction (approx. 1-10 months)	1) FastPrep Lysing Matrix A (MP Biomedicals, 116910050-CF) with FastPrep-24 bead beater (6 m/s, 40s, 2x) and centrifugation (13200 rpm, 5min, 1x), repeated overall 2x, 2) ammonium acetate impurities precipitation and centrifugation (13200 rpm, 15min, 4˚C, 2x), 3) overnight (4˚C) isopropanol DNA precipitation, 4) centrifugation (13200 rpm, 30min, 4˚C) and successive ethanol washes, 5) 100 µL sterile PCR buffer addition	No sampling or analysis controls.	No DNA quantification

Site	Filter Type	Sample Preservation and Storage	DNA Extraction Method	Sampling and Extraction Controls	DNA Quantification
MI	0.2 µm polycarbonate membrane filter disks (EMD Millipore, GTTP04700)	Filters aseptically transferred to 2 mL sterile screw top tubes and stored at -80˚C until extraction	FastDNA SPIN kit (MP Biomedicals, 116540600-CF) with 2 minutes of bead beating with a Bio Spec Mini bead beater instead of the FastPrep step.	Trip, environmental, filter, and filtration set-up negative controls collected during each sampling event. An extraction negative control was included for each extraction session. Positive Legiolert controls per lot. A negative Legiolert control was included for each sampling event.	Qubit dsDNA High Sensitivity assay kit with a Qubit 2.0 fluorometer (Thermo Scientific)
VA	0.2 µm polycarbonate membrane filter disks (EMD Millipore, GTTP04700)	Filters aseptically transferred to 2 mL sterile screw top tubes and stored at -20˚C until extraction	Filters frozen at VT; FastDNA Spin Kit with FastPrep Homogenization	Sampling negative control included during each sampling day. DNA extraction negative control (unused filter) included in each extraction session. Legiolert positive control (manufacturer supplied) and negative control (autoclaved tap water) per lot.	No DNA quantification
MA	NA				
CA	0.22 µm polyethersulfone cartridge filters (EMD Millipore, Z359912)	Filters aseptically transferred to 50 mL sterile screw top tubes and stored at -80˚C until extraction	Modified DNeasy Power Water Kit (QIAGEN, 14900-100-NF). Protocol detailed in dx.doi.org/10.17504/protocols.io.66khhcw.[2]	Environmental and field controls included each sampling day. Filter control included in each extraction batch	Qubit dsDNA High Sensitivity assay kit with a Qubit 4 fluorometer (Thermo Scientific)
CH	NA				

NA: Sample DNA not collected.

Table S6. Quantitative polymerase chain reaction (qPCR) and droplet digital PCR (ddPCR) primers, probes, and standards.

Lab	Sites	Analysis Method	Assay	Gene Target	Amplicon Length (bp)	Forward Primer (5'-3')	Reverse Primer (5'-3')	Probe (5'-3')	Standard Sequence (5'-3')
A	VA, WV, IN, OH, CA	qPCR	Nazarian et al. 2008[1]	*mip*	79	LmipF: 5'-AAAGGCATGCAAGACGCTATG-3' (21 bp, IDT)	LmipR: 5'-GAAACTTGTTAAGAACGTCTTTCATTTG-3' (28 bp, IDT)	LmipP: 5'-FAM-TGGCGCTCAATTGGCTTTAACCGA-BHQ2-3' (24 bp, IDT)	5'-AGCTGTCAGCACTACTAACTTGCGGTCAGTAAAGGCATG CAA GAC GCT ATG AGT GGC GCT CAA TTG GCT TTA ACC GAA CAG CAA ATG AAA GAC GTT CTT AAC AAG TTT CTG CAT GAT CTA CGT GCG TCA CAT GCA GTA C-3' (139 bp, gBlock, IDT)
B	AZ								
C	PA	ddPCR	Wullings et al., 2011[3]	*mip*	120	LpneuF: 5'-CC GATGCCACATC ATTAGC-3' (19 bp, IDT)	LpneuR: 5'-CCAATTGAGC GCCACTC ATAG-3' (21bp, IDT)	None	5'-CCGATGCCACATCATTAG CTACAGACAAGG ATAAGTTGTCTTATAG CATTGGTGCCGATTTGGGGAAGAAT TTT AAAAATCAAGG CATAGATGTTAATCCGGAAGCAATG GC TAAAGGCATGCAAGACGCTATGAGT GGCGCTCAATTGG-3' (150 bp, gBlock, IDT)
D	QC	qPCR	Bio-Rad's iQ-Check Quanti Lp real-time PCR kit (cat. no. 3578103) proprietary assay						Proprietary kit standards
E	MI	qPCR	Nazarian et al. 2008[1]	*mip*	79	LmipF: 5'-AAAGGCATGCAAGACGCTATG-3' (21 bp, IDT)	LmipR: 5'-GAAACTTGTTAAGAACGTCTTTCATTTG-3' (28 bp, IDT)	None	5'-AGCTGTCAGCACTACTAACTTGCGGTCAGTAAAGGCA TG CAA GAC GCT ATG AGT GGC GCT CAA TTG GCT TTA ACC GAA CAG CAA ATG AAA GAC GTT CTT AAC AAG TTT CTG CAT GAT CTA CGT GCG TCA CAT GCA GTA C-3' (139 bp, gBlock, IDT)

Table S7. Quantitative polymerase chain reaction (qPCR) and droplet digital PCR (ddPCR) protocols.

Lab	Method	Master Mix	Instrument	Std curve range	Reaction Mix	Cycling conditions	Replicates
A	qPCR with Nazarian et al. 2008[1]	2X SsoFast Probes Supermix (Bio-Rad, cat no. 1725230)	Bio-Rad CFX96 real-time	$5 – 10^7$ gc/rxn	10 µL reactions: 5 µl of master mix, 250 nM of forward and reverse primers, 93.75 nM probe, and 1 µL of DNA template.	95ºC for 2 min, 40 cycles of 95ºC for 5 s and 60ºC for 10 s	3x
B	qPCR with Nazarian et al. 2008[1]	SSO Fast EvaGreen (Bio-Rad, cat no. 1725200)	Bio-Rad CFX96 real-time	$30 – 10^7$ gc/rxn	25 µL total reaction volume: 12.5 µL universal probe mix, 1.25 µL - 10 µM forward primer (final conc 500 nM), 1.25 µL - 10 µM reverse primer (final conc 500 nM), 0.6 µL - 10 µM probe (final conc 250 nM), 6.4 µL water, 3 µL DNA template	95ºC for 2 min, 40 cycles of 95ºC for 5s, 60ºC for 30s, 72ºC for 30s	3x
C	ddPCR with Wullings et al., 2011[3]	EvaGreen Supermix (Bio-Rad, cat no. 1864034)	Bio-Rad ddPCR QX200 Droplet Generator, C1000 Touch thermocycler, QX200 Droplet Reader	N/A	22 µL reactions: 11 µL of master mix, 0.44 µL of 10 µM forward and reverse primers (final conc 0.2 µM), 0.55 µL of 50 mg/mL BSA (Invitrogen, final conc 0.625 mg/mL, 2 µL DNA template, 7.57 µL water	95ºC for 5 min, 45 cycles of 95ºC for 30 s, 57ºC for 1 min, 72ºC for 1 min, 4ºC for 5 min, 90ºC for 5 min	N/A
D	qPCR Bio-Rad's iQ-Check Quanti Lp real-time PCR kit (cat. no. 3578103)		Rotor-Gene Q QIAGEN	$10^1 – 10^4$ gc/rxn	50 µL total rxn volume: 45 µL amplification mix, 5 µL extracted DNA in sterile PCR buffer	95ºC for 15 min, 50 cycles of 95ºC for 15s, 57ºC for 30s, 72ºC for 30s, 72ºC for 15 min	2x
E	qPCR with Nazarian et al. 2008[1]	Fast EvaGreen w/ low ROX (2x, Biotium, cat. no. 31014)	Applied Biosciences QuantStudio 3	$10^1 – 10^8$ gc/rxn	10 µL total rxn volume: 5 µL of master mix, 0.5 µL of 10 µM forward and reverse primers (final concentration 0.2 µM), 0.625 µL of 25 mg/mL BSA (Invitrogen, final concentration 0.625 mg/mL), 3.25 µL water, and 1 µL DNA template	95ºC for 2 min, 40 cycles of 95ºC for 5s, 60ºC for 30s, 72ºC for 30s	3x

Table S8. Quantitative polymerase chain reaction (qPCR) and droplet digital PCR (ddPCR) standard curve parameters.

Site	Plate	Y-Intercept	Efficiency (%)	R Squared
PA	1	ddPCR		
	2			
	3			
	4			
MI	2	36.3	97.6	0.994
	3	38.0	89.6	0.999
	6	36.8	97.4	0.999
	7	37.2	97.9	0.999
	9	37.7	90.8	0.997
	10	39.1	87.4	0.994
AZ	1	39.9	98.9	0.998
QC	1	36.6	115.0	0.992
	2	37.4	115.0	0.995
CA & WV (run by Lab A)	6	44.3	83.8	0.982
	8	44.4	83.6	0.980
IN & OH (run by Lab A)	7	43.9	85.8	0.988
Nonquantitative plates				
VA	1	50.3	62.6	0.970
CA & WV (run by Lab A)	1	44.7	75.1	0.982
	2	46.3	69.2	0.986
	3	43.9	93.4	0.935
	4	44.6	86.6	0.940

Table S9. Quantitative polymerase chain reaction (qPCR) and droplet digital PCR (ddPCR) limit of detection (LOD) and lower limit of quantification (LLOQ) testing results in gene copies per reaction (gc/rxn).

Site	Run By	Equivalent Volume (mL)	LOD (gc/rxn)	LLOQ (gc/rxn)
AZ	Lab B	60	30	30
QC (plate 1)	Lab D	34.5-48	1.0	16.9
QC (plate 2)			1.0	19.7
MI	Lab E	0.1-14.7	10.2	20.4
VA	Lab A	1.0-1.3	100	100
CA				
WV				
IN & OH				
PA	Lab C	15.5-21.1	6.1	6.1

Table S10. Physicochemical results summary

Parameter	Median Result (Count)					
		By Disinfectant			By *L. pneumophila* Culture Result	
	All Samples	Free Chlorine	Mono-chloramine	None	*L. pneumophila* Positive	*L. pneumophila* Negative
Chlorine Residual (mg/L as Cl_2)	0.05 (n=182)	<0.05 (n=113)	0.28 (n=69)	---	<0.05 (n=46)	0.05 (n=136)
pH	8.4 (n=181)	8.2 (n=112)	9.1 (n=69)	---	8.6 (n=46)	8.3 (n=135)
Temperature (°C)	25 (n=182)	26 (n=113)	23 (n=69)	---	29 (n=46)	23 (n=136)
Dissolved Oxygen (mg/L)	8.1 (n=90)	7.0 (n=58)	9.0 (n=32)	---	6.5 (n= 21)	8.6 (n=69)
Electrical Conductivity (µS/cm)	288 (n=95)	300 (n=63)	100 (n=32)	---	301 (n=27)	276 (n=68)

Table S11. Results of the generalized linear mixed effects model fit by maximum likelihood (Laplace Approximation) for free chlorine samples with associated physicochemical measurements and building characteristics.

Formula and Data	Formula: lp_pos ~ cl_tot_mgl + building_age + temp_c + pH + (1 | building_id) Data: glm1 AIC BIC logLik deviance df.resid 74.4 90.7 -31.2 62.4 106
Scaled Residuals	Min 1Q Median 3Q Max -2.7575 -0.1778 -0.0705 0.2500 5.6366
Random Effects	Groups Name Variance Std.Dev. building_id (Intercept) 11.48 3.388 Number of obs: 112, groups: building_id, 10
Fixed Effects	Estimate Std. Error z value Pr(>|z|) (Intercept) -21.66084 14.70340 -1.473 0.141 cl_tot_mgl -0.29197 4.90055 -0.060 0.952 building_age -0.03532 0.04002 -0.882 0.378 temp_c 0.03244 0.06311 0.514 0.607 pH 2.38928 1.63570 1.461 0.144
Correlation of Fixed Effects	(Intr) cl_tt_ bldng_ temp_c cl_tot_mgl -0.077 building_ag -0.366 0.082 temp_c -0.182 -0.047 0.084 pH -0.980 0.041 0.263 0.054

3. Supplemental Equations

Conversion of limit of detection (LOD) and lower limit of quantification (LLOQ) to gene copies per liter

qPCR/ddPCR LOD and LLOQ were converted to gene copies per liter on a per sample basis, as shown in Equation S1.

Equation S1. Conversion of gene copies per reaction to per liter.

$$\frac{gc}{rxn} \times \frac{rxn}{template\ vol\ (\mu L)} \times \frac{dilution\ factor}{1} \times \frac{extraction\ elution\ vol\ (\mu L)}{sample\ vol\ (mL)} \times \frac{1{,}000\ mL}{L} = \frac{gc}{L}$$

Equation S2. Generalized linear mixed effects model input for free chlorine samples with associated physicochemical measurements and building characteristics

```
output<-glmer(formula = lp_pos ~cl_tot_mgl + building_age+ temp_c+
pH+(1|building_id), data=glm1, family=binomial)
```

Where

lp_pos: binomial vector where 0= *L. pneumophila* culture-negative and 1= *L. pneumophila* culture-positive

cl_tot_mgl: total chlorine result in mg/L as Cl_2

building_age: age of the building in years

temp_c: sample temperature in degrees Celsius

pH: sample pH

building_id: the unique identification name assigned to each building

4. Supplemental Text

Text S1. Quantitative polymerase chain reaction (qPCR) and droplet digital PCR (ddPCR) inhibition testing summary

All laboratories performing qPCR analyses were instructed to perform inhibition testing by analyzing a subset of samples at multiple dilutions. Execution of inhibition testing varied by laboratory and is summarized below.

Laboratory A

Laboratory A analyzed samples from Sites IN, OH, WV, VA, and CA. A subset of samples (n=12) were run undiluted as well as diluted at 1:4, 1:10, and 1:20 to assess inhibition. All samples were then processed at a 1:10 dilution based on the dilution that resulted in the highest concentrations during inhibition testing.

Laboratory B

Laboratory B analyzed samples from Site AZ. All samples were tested for the 16S rRNA target to confirm the extraction process was successful and to determine if samples were inhibited. Both undiluted and 1:10 diluted samples were tested for the 16S rRNA gene target, and the difference in their Cq was calculated. If the Cq difference did not fall in the range of 2 to 4 Cq, the samples were considered to have PCR inhibition. Samples with PCR inhibitors were subjected to a 10-fold dilution while testing for the *Legionella mip* gene target. All other samples were tested without any dilution with a full standard curve for each plate.

Laboratory C

Laboratory C analyzed samples from Site PA using ddPCR. Although ddPCR is less susceptible to inhibitors than qPCR, reactions included 0.625 mg/mL BSA to minimize inhibition. No separate inhibition testing was conducted, and samples were processed undiluted.

Laboratory D

Laboratory D analyzed samples from Site QC. For QC-3 building, all samples (including controls and standards) from plate 1 (n=16) and plate 2 (n=7) were tested for qPCR inhibition according to Bio-Rad's iQ-Check Quanti *L. pneumophila* real-time PCR kit (cat. no. 3578103) user guide. Briefly, inhibition was considered if the Cq sample is higher than the addition of the standards (n=4) mean and three times their standard deviation (Cq > mean Cq QS + 3*sigma). Among all samples, no inhibition was detected during the amplification process.

Laboratory E

Laboratory E analyzed samples from Site MI. Of the 19 samples (excluding controls) analyzed using qPCR, all were analyzed undiluted and at least one dilution. If inhibition was observed based on the delta Cq between the undiluted samples and the first dilution, additional dilutions were performed. Dilutions used included 1:2, 1:5, 1:10, 1:100, 1:1,000, and 1:10,000. The majority of samples did not amplify at any dilution.

5. References

(1) Nazarian, E. J., Bopp, D. J., Saylors, A., Limberger, R. J., & Musser, K. A. (2008). Design and implementation of a protocol for the detection of *Legionella* in clinical and environmental samples. *Diagnostic Microbiology and Infectious Disease*, *62*(2), 125–132. https://doi.org/10.1016/j.diagmicrobio.2008.05.004

(2) Vosloo, S., Sevillano, M., & Pinto, A. (2019). Modified DNeasy PowerWater Kit® protocol for DNA extractions from drinking water samples. *Protocols.Io*. https://doi.org/dx.doi.org/10.17504/protocols.io.66khhcw

(3) Wullings, B. A., Bakker, G., & Van Der Kooij, D. (2011). Concentration and diversity of uncultured *Legionella* spp. in two unchlorinated drinking water supplies with different concentrations of natural organic matter. *Applied and Environmental Microbiology*, *77*(2), 634–641. https://doi.org/10.1128/AEM.01215-10