SUPPLEMENTAL INFORMATION (SI)

Section A: qPCR Assay Details

For the preparation of the qPCR standard curve, gBlocks (amplicon synthesized by IDT) for the *mip* target specific to *L. pneumophila* was serially diluted 10-fold using PCR grade nuclease-free water in a clean PCR hood (Air Clean 600 PCR workstation equipped with a 254 nm short-wave UV light) to help prevent contamination. The amplicon contained a defined high number of gene copies (10^{10} gc/µL). The standard curve ranged from 10^{7} to 10^{-1} gc/µL. All standards were run in triplicates and a full standard curve was run with each 96 well plate for quantifying *mip* gene copies in 1 L of collected water samples. Standard curve amplification efficiencies (E) were considered acceptable between 85 to 110 %, slope ~ -3.3, with coefficient of determination/linearity (R^2) values for standard curve linear regression of at least 0.98 for each plate. To minimize qPCR contamination, DNA extraction and qPCR setup were performed in separate laboratory spaces. The Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) were followed while performing the quantification (61,94). Details are described in **Tables SI.1** to **SI.3**.

Supplemental Table SI.1. qPCR assay sequences for the *Legionella pnuemophila mip* gene target (95)

Target	**Reagent**	**Length**	**Final Concentration**	**Sequence (5' to 3')**
Legionella pnuemophila mip	Forward primer	21	500 nM	AAAGGCATGCAAGACG CTATG
	Reverse primer	28	500 nM	GAAACTTGTTAAGAACG TCTTTCATTTG
	Probe	24	250 nM	FAM – TGGCGCTCAATTGGCTT TAACCGA – BHQ*
	Amplicon (gBlock)	89		TGGCTAAAGGCATGCAA GACGCTATGAGTGGCGC TCAATTGGCTTTAACCG AACAGCAAATGAAAGA CGTTCTTAACAAGTTTC AGAAA

*FAM - reporter and the BHQ – quencher for the probe used in this probe-based assay.

Supplemental Table SI.2. qPCR assay thermocycling conditions for the *Legionella pnuemophila mip* gene

Step	**Temp (C)**	**Time**	**Cycles**
Initial Denaturation	95	2 minutes	1
Denaturation	95	5 seconds	39
Annealing	60	30 seconds	
Extension	72	30 seconds	

Supplemental Table SI.3. qPCR performance characteristics for *L. pneumophila* qPCR assay

# Plate	E	R^2	Slope	Y intercept
1	104.6	0.996	-3.217	38.22
2	100.1	0.991	-3.320	38.99
3	107.7	0.990	-3.150	40.84
4	96.9	0.996	-3.399	39.03
5	90.9	0.988	-3.561	40.60
6	95.1	0.994	-3.510	40.83
7	86.2	0.997	-3.705	40.06
8	91.2	0.990	-3.553	40.15
9	86.5	0.990	-3.693	40.57
10	97.5	0.998	-3.383	38.72

Efficiencies between 80 and 115% and R^2 values of 0.98 were required for all qPCR plates containing positive samples

Section B: Recovery Efficiency

This section describes the methods used to evaluate the recovery efficiency for *L. pneumophila* cultures in drinking water from two operational green buildings using both culture and molecular methods.

Growing *L. pneumophila* and initial quantification

L. pneumophila ATCC 33152 aliquot in a sterile conical tube was thawed at room temperature and was vortexed to get a uniform *L. pneumophila* culture in liquid broth. 10 ml buffered yeast extract broth (BYE) was added to four sterile conical tubes. 1 ml from the aliquot tube which had the *L. pneumophila* ATCC 33152 culture was added to the conical tubes with BYE. It was mixed gently and incubated for 72 hours (3 days) at 37-39 C for regrowing the *L. pneumophila*. After 3 days, white cloudy microbial growth was observed. These conical tubes were mixed gently and 500 ul of new liquid regrown culture was pipetted into several 2 ml sterile Eppendorf tubes. 1ml of sterile phosphate buffer saline (PBS) was added and it was centrifuged to form a white pellet at the bottom of the tubes. This pellet was washed twice with PBS to get a cleaner pellet for quantification. After washing, the pellet was resuspended in 500 ul PBS and mixed gently. 50 ul of the pellet solution was pipetted in triplicates in Qiagen power soil DNA extraction kit bead tubes. For the culture-based method, a volume of 50, 5, and 1 ul of resuspended pellet solution was pipetted in triplicates in 100 ml of DI water for performing the IDEXX Legiolert. The initial culture-based quantification results were checked a week after incubating them for 7 days at 39 C. qPCR for the *mip* target gene was performed on the DNA extracted using the Qiagen power soil pro kit. Along with the samples, the standard curve and NTCs were plated for accurate quantification. Initial input quantification for *L. pneumophila* concentration was performed by using both qPCR and IDEXX culture methods.

The results were used as the starting concentrations to further test for overall recovery efficiency while processing the drinking water samples.

Sample Collection, Spiking, and Final Quantification

1.1 L of drinking water samples in triplicates were collected from different locations and fixtures from the two operational LEED buildings. We collected cold water samples from the breakroom and mixed water samples from restrooms in triplicates and dechlorinated them using sodium thiosulfate. Each water sample was filtered using a 0.2-micron filter and the filtrate was stored in labeled sterile polypropylene bottles. When the water samples reached room temperature, *L. pneumophila* culture with known quantification was carefully spiked (volumes of resuspended pellet solution same as input volumes in all the samples) and they were mixed well. 100 ml of spiked water samples were processed using IDEXX liquid culture method. The final or output concentration of *L. pneumophila* was quantified to calculate culture-based recovery efficiency. The remaining 1L of spiked water samples were processed for qPCR by filtering them and extracting DNA. The target *mip* gene copies were quantified for the output results. The overall recovery efficiency for the process was calculated using Equation SI.1.

Recovery Efficiency = (Output quantification / Input quantification) × 100..... Equation SI.1

Results for Overall Culture-Based and qPCR-Based Recovery Efficiency

The mean recovery efficiency for the IDEXX culture method was 67% and that for the qPCR was 61%. Results have been tabulated below. The efficiency range for recovery between 30 to 80 % is very common for well-developed assays. Recovery depends on several factors

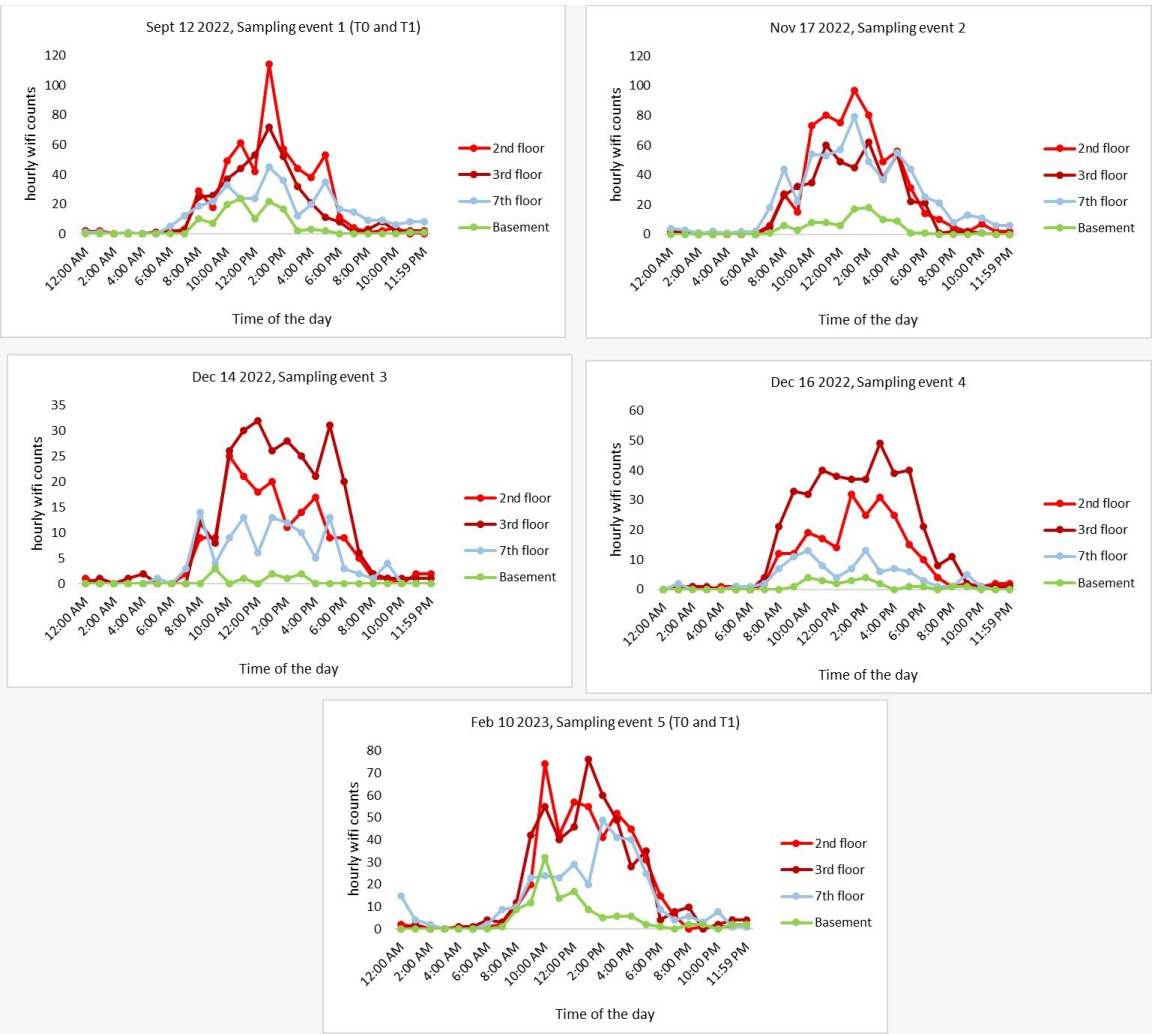
involved in sampling and processing such as the water chemistry, type of DNA extraction kit used, regrowing bacteria, spiking, mixing, purification steps, etc. as there can be losses during many processing steps.

Supplemental Table SI.4. Recovery efficiency

Sample description	Mean of triplicate input	Mean of triplicate output	Processing method	% Recovery	Mean recovery efficiency %	Standard deviation
BDC cold water - breakroom	1070 (MPN/100ml)	590 (MPN/100ml)	IDEXX-culture	55.1	67.1	7.3
BDC mixed water restroom	1070 (MPN/100ml)	720 (MPN/100ml)	IDEXX-culture	67.3		
ISTB4 cold water - breakroom	1070 (MPN/100ml)	790 (MPN/100ml)	IDEXX-culture	73.8		

ISTB4 mixed water - restroom	1070 (MPN/100ml)	770 (MPN/100ml)	IDEXX-culture	72.0		
BDC cold water - breakroom	8.60E+05 (gc/ul)	6.20E+05 (gc/ul)	DNA extraction followed by qPCR	72.1		
BDC mixed water restroom	8.60E+05 (gc/ul)	5.10E+05 (gc/ul)	DNA extraction followed by qPCR	59.3		
ISTB4 cold water - breakroom	8.60E+05 (gc/ul)	5.20E+05 (gc/ul)	DNA extraction followed by qPCR	60.5		
ISTB4 mixed water - restroom	8.60E+05 (gc/ul)	4.60E+05 (gc/ul)	DNA extraction followed by qPCR	53.5	61.3	6.7

Section C: Occupancy Data



Supplemental Figure SI.1. Variation in occupancy (hourly Wi-Fi logins) for each floor during each sampling event from September 2022 to February 2023. Overall occupancy decreases during winter break.

Section D: Statistical Analysis

Supplemental Table SI.5. Wilcoxon signed rank test results pre and post interventions

Water quality parameter	Intervention	P value (reference = 0.05)	Significant difference
Culturable *L. pneumophila* counts before and after flushing for 20 minutes	Flushing in September 2022	0.019	Yes
Culturable *L. pneumophila* counts before and after flushing for 20 minutes combined with increasing temperature setpoint for water heaters	Flushing in February 2023 along with increase in water heater setpoint	0.270	No
Culturable *L. pneumophila* counts for overall building before and after increasing temperature setpoint for water heaters	Water heater setpoint change during December 2022 to January 2023	0.06	No
Culturable *L. pneumophila* counts comparing basement and 2nd floor with 3rd and 7th floor	Water heater setpoint change in December 2022 (assuming the temperature setting for the valve serving the 3rd to 7th floor was at 140 F, observed from high temperature readings for hot water during this period), the other valve settings were not changed until January 2023	0.002	Yes
cATP before and after flushing for 20 minutes	Flushing in September 2022	0.018	Yes
cATP before and after flushing for 20 minutes combined with increasing temperature setpoint for water heaters	Flushing in February 2023 along with increase in water heater setpoint	0.38	No
cATP before and after increasing temperature setpoint for water heaters	Water heater setpoint change during December 2022 to January 2023	0.53	No
L. pneumophila mip gene before and after flushing for 20	Flushing in September 2022	0.51	No

minutes			
L. pneumophila mip gene before and after flushing for 20 minutes combined with increasing temperature setpoint for water heaters	Flushing in February 2023 along with increase in water heater setpoint	0.38	No
L. pneumophila mip gene before and after increasing temperature setpoint for water heaters	Water heater setpoint change during December 2022 to January 2023	0.238	No
Free chlorine before and after flushing for 20 minutes	Flushing in September 2022	0.002	Yes
Free chlorine before and after flushing for 20 minutes combined with increasing temperature setpoint for water heaters	Flushing in February 2023 along with increase in water heater setpoint	0.002	Yes
Free chlorine before and after increasing temperature setpoint for water heaters	Water heater setpoint change during December 2022 to February 2023	0.56	No
DBP (TTHM concentration) before and after flushing for 20 minutes	Flushing in September 2022	0.002	Yes
DBP (TTHM concentration) before and after flushing for 20 minutes combined with increasing temperature setpoint for water heaters	Flushing in February 2023 along with increase in water heater setpoint	0.06	No
DBP (TTHM concentration) before and after increasing temperature setpoint for water heaters	Water heater setpoint change during December 2022 to February 2023	0.06	No
Iron before and after flushing for 20 minutes	Flushing in September 2022	0.002	Yes
Iron before and after flushing for 20 minutes combined with increasing temperature setpoint for water heaters	Flushing in February 2023 along with increase in water heater setpoint	0.002	Yes
Iron before and after increasing temperature setpoint for water heaters	Water heater setpoint change during December 2022 to February 2023	0.58	No
Copper before and after flushing for 20 minutes	Flushing in September 2022	0.60	No
Copper before and after flushing	Flushing in February	0.60	No

for 20 minutes combined with increasing temperature setpoint for water heaters	2023 along with increase in water heater setpoint		
Copper before and after increasing temperature setpoint for water heaters	Water heater setpoint change during December 2022 to February 2023	0.01	Yes
Building Occupancy	Morning vs afternoon	0.002	Yes

Supplemental Table SI.6. Kendall's Tau correlation coefficient

Parameters	Coefficient	Correlation
Occupancy (Wi-Fi log in) VS Culturable *Legionella*	-0.19	Very weak negative association
Free Chlorine VS Culturable *Legionella*	-0.31	Weak negative association
cATP VS Culturable *Legionella*	+0.45	Moderate positive association
qPCR (*mip* gene) VS Culturable *Legionella*	+0.15	Very weak positive association
cATP VS Free Chlorine	-0.48	Moderate negative association
Occupancy VS Free Chlorine	+0.25	Weak Positive association
Temperature VS Free Chlorine	-0.39	Weak negative association
DBP (TTHM) VS Free Chlorine	+0.21	Weak Positive association