Supplementary Material

1. ddPCR Information

Legionella genomic DNA

Five *Legionella* species were obtained from American Type Culture Collection (ATCC®) and are described below. Positive controls using DNA from *L. pneumophila* (ATCC No. 33152), *L. micdadei* (ATCC No. 33218), *L. anisa* (ATCC No. 35292), *L. bozemanii,* (ATCC No. 33217) and *L. longbeachae* (ATCC No. 33462). DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Valencia, CA) and described in great detail in the experimental section. The concentrations were estimated using a UV spectrometry (Nanodrop). Serial dilutions of the stock gDNA into DNAase/RNAase free water were performed to produce up to five ten-fold dilutions. A temperature gradient (52-65°C) was initially run to determine the optimal degree for primer/probe annealing for amplification on each ten-fold dilution. The dilution factor (10^{-5}) that was not saturated of the ddPCR reaction was used for the assay. All gDNA were stored at -20°C. For each ddPCR assay, a freshly thawed aliquot (~20 µl) gDNA was used without further dilution.

Sample DNA

The concentrations were estimated using a UV spectrometry (Nanodrop). DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Valencia, CA) and described in great detail in the experimental section. All samples were stored at -80°C and analyzed by ddPCR within 30 days of extraction. For each ddPCR assay, a freshly thawed aliquot (~60 µl) sample was used without any dilution; afterwards each sample was held in the 4°C refrigerator for up to 48 hours —in the event the assay needed to be re-run; if not, any remaining sample was discarded.

Assay Validation

Each assay was optimized by running an annealing temperature gradient using a Bio-Rad C1000 Touch thermal cycler. The assay format is a duplex reaction, and the details of the experimental set are described in the full body of the manuscript. There was no amplification of either target in buffered solution (phosphate buffered water), nor was there any cross-reactivity with either positive control. The detection limit was determined for all assays by multiplying the minimum number of droplets (3) as well as back calculating the initial and final volume of water collected and assayed. The assays lower detection limit detectable by the ddPCR method is 1.3 GC/100mL when filtering 10L and extracting a concentrated subsample of 10mL. Positive gDNA was spiked to determine any inhibition of the assay and no inhibition was observed for either of the duplex assays.

Data Analysis (Partition classification method)

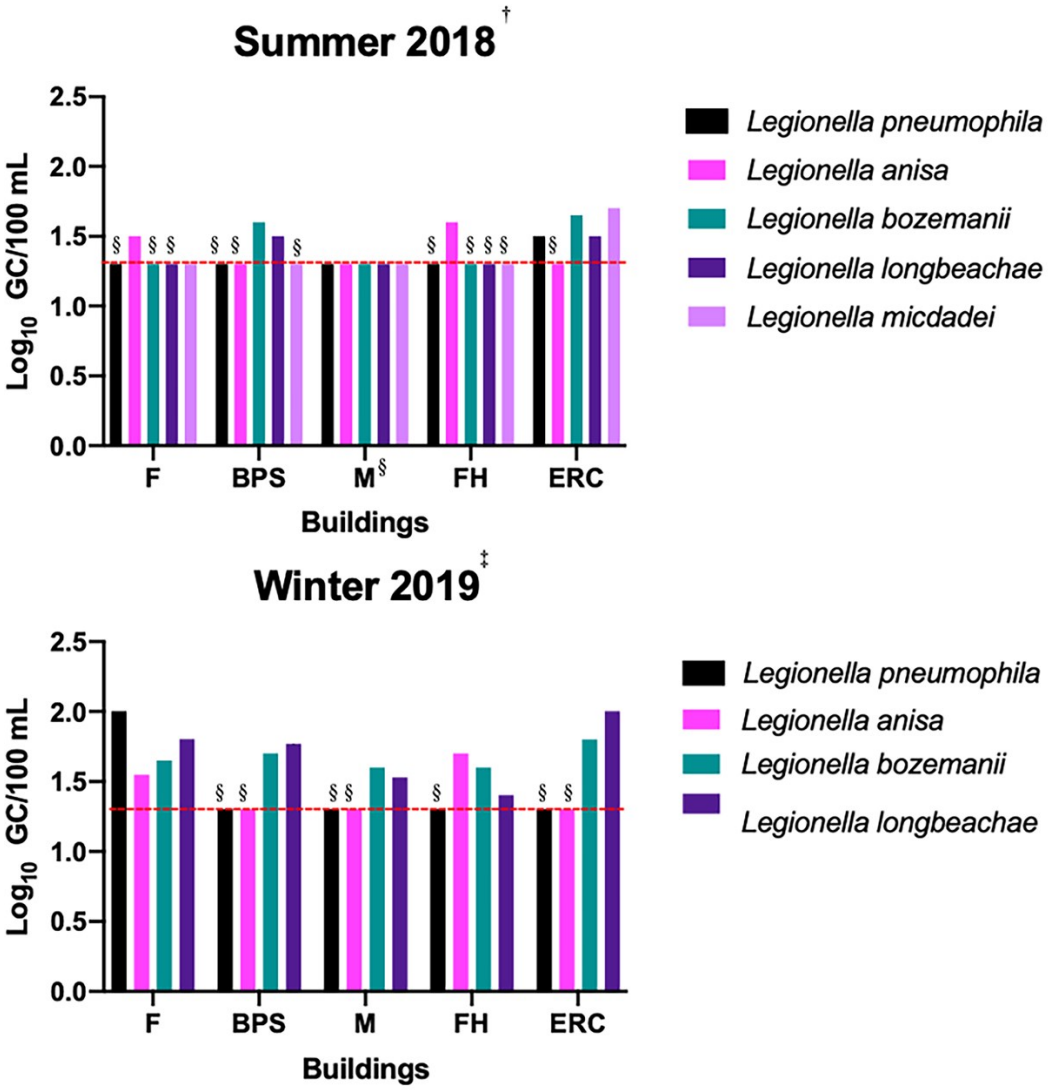
The analysis software that was used was QuantaSoft v.1.7.4.0917. The threshold was manually set, which was based off of the positive and negative controls; the classification mode was a two-color plot (channel 1 and channel 2). There were at least three positive and negative controls to match triplicate reactions per sample. Repeatability of each technical replicate from the biological samples varied across assays, ranging from 33 to 100%. Replication of the entire experiment process (biological replicates from each site, including all preanalytical steps such as collection, extraction, and measuring) was not evaluated.

Table 1. ddPCR Experimental Information

ITEM TO CHECK	PROVIDED
	Y/N
1. SPECIMEN	
Detailed description of specimen type and numbers	Y
Sampling procedure (including time to storage)	Y
Sample aliquotation, storage conditions and duration	Y
2. NUCLEIC ACID EXTRACTION	
Description of extraction method including amount of sample processed	Y
Volume of solvent used to elute/resuspend extract	Y
Number of extraction replicates	Y
Extraction blanks included?	Y
3. NUCLEIC ACID ASSESSMENT AND STORAGE	
Method to evaluate quality of nucleic acids	Y
Method to evaluate quantity of nucleic acids (including molecular weight and calculations when using mass)	N
Storage conditions: temperature, concentration, duration, buffer, aliquots	Y
Clear description of dilution steps used to prepare working DNA solution	N
4. NUCLEIC ACID MODIFICATION	
Template modification (digestion, sonication, pre-amplification, bisulphite etc.)	N
Details of repurification following modification if performed	N
5. REVERSE TRANSCRIPTION	N
cDNA priming method and concentration	N
One or two step protocol (include reaction details for two step)	N
Amount of RNA added per reaction	N
Detailed reaction components and conditions	N
Estimated copies measured with and without addition of RT*	N
Manufacturer of reagents used with catalogue and lot numbers	N
Storage of cDNA: temperature, concentration, duration, buffer and aliquots	N
6. dPCR OLIGONUCLEOTIDES DESIGN AND TARGET INFORMATION	
Sequence accession number or official gene symbol	Y
Method (software) used for design and *in silico* verification	N
Location of amplicon	Y
Amplicon length	Y
Primer and probe sequences (or amplicon context sequence)**	Y
Location and identity of any modifications	N
Manufacturer of oligonucleotides	Y
7. dPCR PROTOCOL	
Manufacturer of dPCR instrument and instrument model	Y
Buffer/kit manufacturer with catalogue and lot number	Y
Primer and probe concentration	Y
Pre-reaction volume and composition (incl. amount of template and if restriction enzyme added)	Y
Template treatment (initial heating or chemical denaturation)	Y
Polymerase identity and concentration, Mg++ and dNTP concentrations***	Y
Complete thermocycling parameters	Y
8. ASSAY VALIDATION	
Details of optimisation performed	Y
Analytical specificity (vs. related sequences) and limit of blank (LOB)	Y
Analytical sensitivity/LoD and how this was evaluated	Y
Testing for inhibitors (from biological matrix/extraction)	Y
9. DATA ANALYSIS	
Description of dPCR experimental design	Y
Comprehensive details negative and positive of controls (whether applied for QC or for estimation of error)	Y
Partition classification method (thresholding)	Y
Examples of positive and negative experimental results (including fluorescence plots in supplemental material)	N
Description of technical replication	Y
Repeatability (intra-experiment variation)	Y
Reproducibility (inter-experiment/user/lab etc. variation)	N
Number of partitions measured (average and standard deviation)	N
Partition volume	N
Copies per partition (λ or equivalent) (average and standard deviation)	N
dPCR analysis program (source, version)	Y
Description of normalisation method	N
Statistical methods used for analysis	Y
Data transparency	

2. Pathogenic *Legionella* Species in All Five Buildings

Supplementary Figure 1. Presence of Pathogenic *Legionella* Species in the Five Buildings. Bars Reflect All Measurements Collected at Various Sampling Sites (described below in footnote). Dashed Line Represents the Detection Limit (1.3 Log_{10} GC/100 mL). Samples With No Signal are Reported as the Detection Limit.



† In the summer for the composite samples Buildings F and FH only had one species detected (*L anisa*) and building M had no detects.Building BPS was positive for both *L. bozemanii,* and *L. longbeachae* and ERC was positive for *L. pneumophila, L. bozemanii, L. longbeachae* and *L. micdadei* ‡ In the winter sampling hot water taps were positive in F, BPS, M, FH, and ERC for *L. pneumophila, L, anisa, L. bozemanii,* and *L. longbeachae.* The cold-water taps were positive in F, BPS, M, and FH for *L, anisa, L. bozemanii,* and *L. longbeachae.* § Dotted line is the detection limits which corresponds to the non-detect samples.