Temperature diagnostic to identify high risk areas and optimize *Legionella pneumophila* surveillance in hot water distribution systems

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Abstract

Legionella pneumophila is frequently detected in hot water distribution systems and thermal control is a common measure implemented by healthcare facilities. A risk assessment based on water temperature profiling and temperature distribution within the network is proposed, to guide effective monitoring strategies and allow the identification of high risk areas. Temperature and heat loss at control points (water heater, recirculation, representative points-of-use) were monitored in various sections of five healthcare facilities hot water distribution systems and results used to develop a temperature-based risk assessment tool. Detailed investigations show that defective return valves in faucets can cause widespread temperature losses because of hot and cold water mixing. Systems in which water temperature coming out of the water heaters was kept consistently above 60°C and maintained above 55°C across the network were negative for *Legionella* by culture or qPCR. For systems not meeting these temperature criteria, risk areas for *L. pneumophila* were identified using temperature profiling and system's characterization; higher risk was confirmed by more frequent microbiological detection by culture and qPCR. Results confirmed that maintaining sufficiently high temperatures within hot water distribution systems suppressed *L. pneumophila* culturability. However, the risk remains as shown by the persistence of *L. pneumophila* by qPCR.

Key words

Legionella pneumophila, premise plumbing, viable but not culturable (VBNC), heat treatment, temperature profile, culturability.

Highlights

- Temperature profiles were generated for hot water distribution systems points-of-use
- Risk assessment based on temperature profile results at control points was developed
- *L. pneumophila* positive areas were predicted using the risk assessment tool
- A temperature diagnostic flowchart is proposed to identify *L. pneumophila* risk areas

1. INTRODUCTION

Legionella pneumophila (*Lp*) is an opportunistic pathogen that can proliferate in hot water distribution systems (HWDS) of large buildings, such as health care facilities (HCFs), where it can cause waterborne nosocomial pneumonias. Although its optimal growth temperature lies between 25 and 42°C (Yee and Wadowsky 1982), *Lp* has been isolated from water systems at temperatures up to 60°C (Martinelli et al. 2000), and in cold water systems with temperatures below 20°C (Arvand et al. 2011). The presence of *Lp* in HCFs water systems is well demonstrated, with reports of 10 to 50% positive hot water samples taken from taps and showers in Europe and the United States (Arvand et al. 2011, Bargellini et al. 2011, Martinelli et al. 2000, Serrano-Suarez et al. 2013, Stout et al. 2007). Risk characterization of water sources remains uncertain because of the lack of reliable dose-response models (Buse et al. 2012) and therefore the difficulty to define an acceptable level of *Lp* contamination that would minimize risk. While the level of positivity for *Legionella* in health care facilities (HCF) HWDS has been proposed as a reliable predictive risk factor (Best et al. 1983, Lin et al. 2011), the specificity and sensitivity of the 30% positivity cut-off point has been recently questioned (Allen et al. 2012, Allen et al. 2014, Pierre et al. 2014).

Control of *Legionella* risks in health care facilities (HCFs) is addressed and regulated through guidance documents (Bartram et al. 2007, BSR/ASHRAE 2013, CDC 2003, HSE 2013, République Française 2010a). System characterization and environmental monitoring are among the first steps to establish a water safety plan or to evaluate the

87 operational risk in hot water distribution systems (HWDSs), especially in HCFs
88 (BSR/ASHRAE 2013, Department of Health (DH) and Estates and Facilities Division 2006,
89 République Française 2010b, WHO 2011). Recent guidelines stress the need to properly
90 manage hydraulics to ensure homogeneous temperature and biocidal control in all
91 areas of the HWDS (CSTB 2012), and system balancing under varying demand should be
92 verified.
93
94 Although a multitude of possible system architectures are encountered, a simplified
95 schematic of a hot water distribution system (HWDS) can be established (Fig. 1) and
96 should include: the number and characteristics of key systems components such as the
97 calorifiers, reheating units and reservoirs, the distribution systems including principal,
98 subordinate and tertiary flow and return loops and point-of-use devices (tertiary
99 terminal end). A schematic and characterization of each HWDS within a premise must
100 be established independently (BSR/ASHRAE 2013, HSE 2013, République Française
101 2010a). This data is the foundation for interpreting monitoring results and identifying
102 high risk areas.
103
104 A summary of the key elements from selected regulations and guidelines to implement
105 temperatures control of *Lp* in large buildings, and when available, in HCFs is provided as
106 supplementary material (Table S1). Approaches to control *Lp* in hot water distribution
107 systems (HWDSs) vary considerably, but all guides include objectives or obligations for
108 optimal operating temperatures at critical points in the distribution systems. Also

109 commonly specified are construction and operational standards, such as minimizing
110 stagnation (recirculation loops, elimination of hydraulic and physical dead ends, etc.),
111 recommendations on the use of devices and materials not promoting bacterial
112 proliferation (construction material, flow, temperature, etc.) and requirements for
113 microbiological monitoring in relation to pre-established criteria that define corrective
114 actions.
115
116 In France, recently strengthened regulations determine mandatory minimum
117 temperature and *Legionella* monitoring at defined critical control points: 1) hot water
118 outlet and reservoir when present; 2) return loop; and 3) representative points-of-use
119 considered at risk (farthest from the water heater or serving vulnerable patients) but
120 the number of sampling points to be monitored is not specified (République Française
121 2005, 2010a, b, Table S1). It is recommended that temperatures be monitored daily or
122 continuously at hot water heater outlets and at each return loops, and weekly at service
123 points in HCFs. Temperature measurements at points of use are conducted on flushed
124 samples (2-3 min). In the United Kingdom, a risk management approach is proposed,
125 with recommended preventive measures including system maintenance, elimination of
126 stagnation or dead zones, reduction of aerosol formation, maintenance of adequate
127 temperatures and use of materials unfavorable to biofilm development (Department of
128 Health (DH) and Estates and Facilities Division 2006, HSE 2013). Temperature control
129 regimen is presented as the preferred initial approach for *Legionella* control (Table S1).
130 Minimal monthly temperature monitoring is specified at control points including water

heater outlet, return loops and sentinel taps. Sentinel taps include representative at-risk taps as well as the first and last taps of each return loops. The use of continuous temperature monitoring is recommended for the water heater outlets and the return loops. In addition, temperature at the tap should be monitored annually on a rotating basis covering 20% of taps yearly, to ensure the whole system is meeting required temperatures for *Legionella* control. It is not permissible to shut down pumped recirculation as it would lead to the loss of the required system temperatures. *Legionella* monitoring is not prescribed unless target temperatures cannot be achieved; however it is recommended in areas with highly vulnerable patients. Weekly flushing for several minutes is recommended for low usage taps.

Although all available regulations and guidelines provide information on various aspects of the implementation of a successful temperature control regimen, there is no consistent guidance on key elements such as the selection of sentinel points, the incorporation of *Lp* monitoring and the interpretation of the temperature monitoring results. Reports on the efficacy of the implementation of temperature control in health care facilities (HCFs) reveal limited success (Arvand et al. 2011, Bargellini et al. 2011, Blanc et al. 2005, Darelid et al. 2002, Hruba 2009, Lee et al. 2011, Serrano-Suarez et al. 2013). Nevertheless, adjusting the temperature at the heater outlet to ensure water temperatures greater than 50-55^{o}C at distal outlets can be highly effective in reducing the proportion of positive swabs or water samples (Arvand et al. 2011, Blanc et al. 2005, Ezzeddine et al. 1989). Moreover, areas consistently positive for *Lp* were associated with

153 poor hot water recirculation leading to temperature losses (Blanc et al. 2005). In most
154 case studies, the actual conditions of application of the temperature control regimen
155 are poorly documented with some information on temperatures only available for the
156 water heater and return. The efficacy of temperature control regimens must be
157 assessed by its ability to suppress *Lp* growth in the distal areas, as distal growth is highly
158 significant (Cristina et al. 2014, Serrano-Suarez et al. 2013). On the other hand, there is
159 increased risk of scalding for temperatures higher than 50°C at the tap (Moritz and
160 Henriques 1947). Some countries specify maximum temperatures at the point-of-use to
161 avoid scalding (Table S1), but newly updated regulation in United Kingdom require a risk
162 assessment comparison between the risk of scalding and the risk of infection before
163 limiting the hot water temperature below 50°C, a risk factor for *Legionella* proliferation.
164
165 Although the critical elements of temperature control in guidelines and regulations to
166 reduce *Legionella* risks in HWDSs rely on scientific evidence and application experience,
167 the detailed implementation, especially the selection of critical control points and
168 monitoring requirements, most often reflect economic constraints. In addition,
169 significant discrepancies exist between proposed modalities of implementation and
170 management. The objectives of the present study were to: (1) demonstrate the
171 potential of detailed temperature profiling to identify areas at risk of *Lp* in the hot water
172 distribution systems (HWDSs) of five health care facilities (HCFs); (2) identify effective
173 monitoring strategies and guidance to conduct temperature profiling and interpret

monitoring results; (3) propose a risk characterisation approach based on temperature diagnostic at critical control points.

2. MATERIALS AND METHODS

2.1. Hot water system characterization

Five hot water distribution systems (HWDSs) were analyzed. Systems 1 to 4 are smaller systems within a 7-story general hospital facility of 255 beds using conventional electric water heater being fed chlorinated ground water. System 5 has a larger flash system feeding a ten-story 450 bed children's hospital fed by surface filtered chlorinated water. A survey of the different HWDSs and connected units was first completed.

The principal flow and return loop of each system was sampled at the water heater outlet, in the principal return loop and prior to the return point into the water heater. The sampling ports were seldom used and were flushed prior to sampling to ensure no stagnant water from the sampling port would be collected. The sampling port was cleaned with ethanol and sterilized MilliQ water. Two samples were collected at each point: 1) 2L in sterile polypropylene bottles with sodium thiosulfate (final concentration of 1.1mg/L) microbiological analysis and 2) 250 mL for pH, temperature, chlorine and conductivity measurements. Municipal water feeding the hot water systems was sampled following the same protocol. In addition continuous temperature monitoring was conducted on 3 subordinate return loops for system 5, using a Datalogger (RDXL4SD 4-Channel, Omega, Qc, Canada).

195

196 **2.2. Temperature profiling and water sampling at points-of-use**

197 Sentinel taps where sampling was performed were selected based on the following
198 criteria: representative of different building levels, some at the far end and preferably in
199 areas serving vulnerable patients such as intensive care units, surgical ward, transplant,
200 infectious diseases. All sampling events were conducted between July 2012 and October
201 2013. The first part of the sampling campaign was conducted to establish temperature
202 profiles at each selected sentinel point-of-use in hot water, across all 5 systems between
203 July 2012 and March 2013. A temperature probe was inserted into the water to
204 measure the temperature over a 20 minute period of continuous flow. Each system had
205 a number of taps sampled proportional to the size of the system. Systems 1 to 5 had
206 respectively 3, 6, 3, 7 and 36 taps sampled. The temperature profiles could not be
207 generated for 1 mitigated tap in system 1, 3 in system 2 and 1 in system 3. In addition,
208 three taps from system 5 were selected for a repeat temperature profile sampling.
209 Residual chlorine was measured onsite (Pocket Colorimeter^{TM} II, Hach, USA) for all
210 samples.

211

212 The second part of the sampling campaign was conducted to evaluate the presence of
213 *Lp* at the point-of-use. All sentinel points of systems 1 to 4 and 8 sentinel points from
214 system 5 were sampled for microbiological analysis. Sentinel points from system 5 were
215 selected based on temperature profile results. For each sampling point, 3L of hot water
216 were collected without prior flush into sterile polypropylene bottles containing sodium

thiosulfate (final concentration of 1.1 mg/L). Of the 3 liters collected, 1L was used for culture, 1L for qPCR and 1L was collected as extra. This sampling was repeated 4 times at 3 selected sampling points in 2 systems fed by the same source water: a system with no positive sites for *Lp* (system 1) and a system with a high positivity rate (system 4). The 3 control points selected were the water heater outlet, one representative tap and the principal return loop.

2.3. Impact of stagnation

The third part of the sampling campaign was conducted on 2 taps of system 5 to study the impact of stagnation on the detection of *Lp*. Stagnation is defined as the period during which the tap is not used and water remains idle within the piping. One liter of hot water was sampled in sterile propylene bottles with sodium thiosulfate (final concentration 1.1 mg/L) after 1 hour, 1, 2, 3, 5 & 10 days of stagnation. These stagnation times were chosen to represent various situations within a real system: 1h for the time between usage in a patient room; 1-day for patient daily care; 2 and 3 days for areas closed on the weekend (i.e. outpatients clinics); 5 and 10 days for an empty room in between patients or a temporary ward closure. *Lp* concentrations were measured by qPCR as described in section 2.4.

Heat losses during stagnation periods were evaluated in the laboratory, on 81 cm of 1.25 cm diameter copper pipes at room temperature (20°C) without insulation and with insulation: Type 1, 2.54 cm thick fiberglass insulation with PVC jacket (Caltech Isolation,

239 Canada) and Type 2, 0.95 cm thick polyethylene foam insulation (Tundra, Industrial
240 Thermo Polymers Limited, Canada).
241
242 **2.4. Microbiological analyses**
243 Water samples were mixed thoroughly and divided to perform isolation and
244 quantification of *Legionella* spp. and *Lp* by culture and quantitative polymerase chain
245 reaction (qPCR).
246
247 Culture was conducted according to the standard AFNOR NF T90-431 procedure (AFNOR
248 2006). Briefly, 1 liter was filtered on sterile 0.4 µm polycarbonate membranes (47 mm
249 diameter; Maine Manufacturing, LLC), which were then sonicated in 5 ml sterile water
250 at 47 kHz for 1 min (Bransonic, Danbury, USA). Heat treatment (50°C, 30 min), acid
251 treatment (pH=2; 5 min) and combination of both were performed on 3 separate 1 ml
252 aliquots. Samples were plated on GVPC selective agar (Innovation Diagnostics Inc.) and
253 incubated at 36°C for 10 days. Typical colonies that developed after 4 to 10 days were
254 sub cultured on confirmation plates for 2 to 4 days at 36°C. Resulting colonies that
255 developed on BCYE agar, but neither on blood agar nor on BCYE without cysteine were
256 considered as *Legionella* spp. Confirmation for *Lp* was conducted using the *Legionella*
257 latex test (DR0800, OXOID Limited). The calculated detection limit for the culture
258 method was 50 CFU/L for both *Legionella* spp. and *L. pneumophila*.
259

Quantification by qPCR was performed on a Corbett Rotorgene 6000 using the iQ-Check Quanti *L. pneumophila* kit (Bio-Rad, Mississauga, Canada) with the following protocol: 15 min initial denaturation at 95°C followed by 50 cycles with denaturation at 95°C for 15 s, annealing at 57°C for 30 s, elongation at 72°C for 30s and final elongation for 15 min at 72°C (Bonetta et al. 2010). An internal control and four DNA standards ranging between 19 and $3.9x10^4$ genomic units (GU) were supplied with the kit. Sterilized water was used as negative control. DNA extraction was performed directly on filters using a bead beating method adapted from Yu and Mohn (1999). Briefly, 1L was filtered on 0.45 µm mixed cellulose ester and the filter was inserted into an extraction tube containing a garnet matrix and one 1/4-inch ceramic sphere (Lysing Matrix A, MP Biomedicals, Solon, USA). Lysing buffer was added to each tube prior to the bead beating step performed on a FastPrep MPBio-24, followed by ammonium acetate precipitation and successive ethanol washes.

2.5. Statistical analysis

Statistical analyses were performed with Statistica10 (StatSoft). A one-way analysis of variance (one-way ANOVA) was used to evaluate differences between the 3 control points sampled in systems 1 and 4 during the repeat sampling. A t-test was used to detect differences between the two taps sampled at various stagnation times in system 5. Significance level was set at $p = 0.05$.

3. RESULTS & DISCUSSION

3.1. General system characterization

Systems 1, 3, 4 & 5 presented a multiple vertical subordinate flow and return loop configuration feeding in average three devices per story. System 2 was a simplified horizontal architecture with only few vertical pipes feeding water to horizontal subordinate flow and return loops (Fig. 1). There is no reported evidence showing that the vertical or horizontal configuration is a determining factor for the risk of contamination. Other factors including hot water temperature, effective recirculation in the subordinate loop, the presence of dead-ends, piping material and water velocity have been identified as risk factors (Health and Safety Executive (HSE) 2013). Nevertheless, it is important to know and document the configuration of a studied system to interpret temperature data collected. With information on the pipe diameter and configuration, the location and relative importance of recirculating and stagnating volumes can be determined providing information to guide monitoring and control strategies. For example, the recirculated volume was approximately 900L, of which 600L in the principal flow and return loop (50 mm diameter) and 300L in the subordinate flow and return loops (10 vertical risers of 25 mm mean diameter). The distal volume in the tertiary terminal end was about 300L (Fig. 1). However, this volume can be minimized if a tertiary return loop is added, leaving only the small connecting volume of less than 150mL per device accounting for a total of 90L of stagnant volume (Fig. 1).

For systems 1 to 4, incoming water had chlorine residual of 0.30±0.03 mg Cl_2/L, pH of 7.77±0.05 and conductivity of 307±29 µS. For system 5, residual chlorine was higher, at 0.5±0.1 mg Cl_2/L, pH of 7.82±0.07 and conductivity of 288±13 µS. There was no additional disinfection treatment in any of the hot water systems studied and mean residual chlorine was 0.04±0.02 mg Cl_2/L for all systems.

3.2. Temperature monitoring

3.2.1. Water heater outlet

Most guidelines specify that target temperatures must be maintained at all times, but seldom do they specify the monitoring requirements of measurement frequency. Periodic temperature readings, even daily measurements, do not provide insurance of temperature maintenance in the hot water distribution system (HWDS), unless the stability of the system's performance has been fully established. Systems seemingly providing water above 60°C based on daily measurements can actually produce lower temperature water for extended periods of time. In fact, the mean temperatures at the water heater outlet for four of the five systems studied were above 60°C, but online temperature monitoring revealed that production temperature was repeatedly below 60°C and reached down to 43°C in some cases (Table 1 and Fig. 2). System 1 consistently produced water above 60°C while systems 2, 3 and 5 regularly produced water below 60°C at certain periods of the day (Fig. 2). For system 3, temperature was monitored weekly by the operators on Saturday mornings during low water demand

323 providing an average of 62.5°C over a period of 24 months (Table 1). Nonetheless, when
324 online monitoring was performed during a typical weekday, mean temperature was
325 lower (57.8°C). It is also interesting to point out that even a very recently installed
326 system (2011) equipped with a flash heating unit was also subject to periodic
327 temperature drops (System 5, Fig. 2). These observations demonstrate the need to use
328 online monitoring to assess the temperature compliance of a HWDS compared to
329 periodic manual readings of temperature. Daily variations in hot water demand in large
330 HCFs with typical peak flow factors of > 6 (Bujak 2010) can influence the temperature at
331 the water heater outlet depending on the system's capacity. The extent and duration of
332 the non-compliance of the hot water outlet temperature set point is important to
333 consider and has been limited to the sporadic short duration (minutes) events in the
334 German technical rules (Table S1).

335

336 3.2.2. Return loops

337 The return loop at the point closest to the water heater is designated as the furthest
338 point from the water heater and continuous temperature monitoring is often
339 recommended (Fig. 1). It is considered as an indicator of the system's capacity to
340 maintain temperatures throughout the hot water distribution system (HWDS). In the
341 five systems studied, the principal return loop temperatures ranged between 50.4 and
342 58.9°C with varying levels of blending from multiple return loops occurring upstream of
343 the principal return control point (Table 1). Continuous monitoring for 2 months at the
344 return loop manifold for combined returns of units 3&5 (45.7°C), units 1&2 (48.0°C),

345 single return for the kitchen (58.1°C) and for unit 3 prior to merging with unit 5 (46.6°C)
346 revealed wide differences compared to the overall combined return loop (53.9°C).
347 Although a regulated control location (Table S1), temperature at the principal return
348 loop is not indicative of the conditions in all subordinate loops within a complex HWDS if
349 the system is not balanced for all water demand conditions. In such cases, it merely
350 represents the mean temperature of the blended recirculated hot water from various
351 sectors of the HWDS. More specifically, it does not provide any information on the
352 actual levels of recirculation and temperature losses in the various sectors of the HWDS
353 and does not in any way confirm efficient recirculation in all subordinate loops. These
354 results suggest the temperature monitoring of subordinate return loops together with
355 the principal return loop as a tool to identify imbalances within a system and as an
356 ongoing system validation measure.
357
358 Heat losses between the water heater outlet and a remote point will occur during
359 stagnation (if recirculation is not effective or shut down for energy conservation
360 purposes) or during circulation in the principal and subordinate flow and return loops.
361 During low demand conditions, recirculation will dictate residence time and drive heat
362 losses. Mean system heat losses were evaluated for each of the five studied systems
363 (Table 1). For three of the five systems, temperature losses between the water heater
364 and the principal return loop mean temperatures exceeded the target of ≤5°C set in
365 several guidelines (Table S1). Heat losses during circulation can be minimized by
366 reducing residence time. Water velocity can be set to meet desired maximum heat

367 losses and general recommendations suggest maintaining a minimal velocity of 0.2 m/s
368 (Blokker et al. 2010, CSTB 2012), which would result in approximately 30 min residence
369 time and 5°C heat losses in large health care facilities (HCF) insulated HWDS. Although
370 insulation minimizes heat losses under flowing conditions, it is not sufficient to maintain
371 high temperatures over prolonged periods of stagnation. Actually, slower heat losses
372 during stagnation may lead to sustained optimal temperatures for *L. pneumophila*
373 growth. Figure 3 shows that temperature decreased from 60°C to below 50°C within 30
374 min in fully insulated copper pipes and within 10 min for non-insulated pipe, both
375 reaching room temperature after 3.5 hours. Periods of stagnation of 30 min or more are
376 expected in the connecting piping upflow of points-of-use and in areas of inefficient
377 recirculation.
378
379 Existing standards and guidelines set design and operational obligations to control heat
380 losses in hot water distribution systems (HWDS) to maintain at minimum target
381 temperatures throughout the HWDS and to meet energy conservation goals, but these
382 are generally only compulsory for new buildings. Recirculation flow rates should be
383 calculated to maintain a $<5^{o}$C system heat loss or to ensure a minimum temperature of
384 50-55°C at the end of the return loop assuming adequate recirculation throughout the
385 system (ASPE 2008). The control points results required to evaluate heat loss goals
386 compliance include the principal and subordinate return loops, the most distant point of
387 the flow loop or all points of the system (Table S1). Monitoring results from the five
388 HWDSs clearly show that the selection of the return loop reference point is critical. Heat

389 loss evaluation from the principal return loop may mask major heat losses in
390 subordinate flow and return loops, as we observed in system 5 with losses ranging from
391 3.5 to 16.3°C when evaluated for single or dual subordinate return loops (Table 1).
392 Indeed, wide differences in temperature can occur between secondary return loops,
393 and thus all return loops should be considered individually. The overwhelming
394 importance given to temperature maintenance has also led to the specific banning of
395 recirculation shutdown in Austria and United Kingdom (Table S1). The nightly shutdown
396 of recirculation for energy conservation purposes is only allowed in two rules
397 (CMMQ/RBQ 2013, DVGW German Technical and Scientific Association for Gas and
398 Water 2004) and only with the demonstration of unobjectionable hygienic conditions.
399 Our results point out that the temperature losses of isolated subordinate loops during
400 stagnation resulting from such shutdowns would quickly generate durable temperature
401 conditions favorable to the growth of *Lp*. More importantly, such shutdowns during low
402 or nil demand conditions expose the whole HWDS, instead of a relatively small volume
403 (1,200L versus 90 to 300L in System 5) to these undesirable temperature conditions.
404
405 3.2.3. Temperature distribution at point-of-use
406 Sequential volume profiling results identify in which sections of the HWDS the heat
407 losses take place, namely the tap and its connecting piping, the secondary piping, the
408 distribution columns and/or the main feeder pipes. Profile variability for a given
409 sampling point at different times and days was found to be small, with overall profile
410 and maximum temperature reached being consistent over time despite variable

411 temperature in the first liter (Fig S1). Temperature profiles obtained on the studied
412 systems are summarized in three groups (Fig. 4), with detailed profiles presented in Fig.
413 S2. Systems 1, 2 and 3 (Fig. 4a) met recommendations for water heater outlet and
414 return loop temperatures, with 86% of points reaching 55°C and all points being above
415 50°C after 2 minutes of flow, indicative of limited stagnant water volumes and effective
416 recirculation. Ideal systems should have no or very little transition and reach equilibrium
417 at recommended temperatures in order to maintain sufficient temperatures within the
418 whole system. Despite reaching equilibrium temperature rapidly (<60s), system 4 could
419 not achieve recommended temperature at the points-of-use with 57% of points never
420 reaching 55°C although all above 50°C, mainly due to the insufficient water temperature
421 at the water heater outlet (Fig. 4b). System 5 shows a longer transition period before
422 reaching temperature equilibrium and is unable to meet 55°C for 47% and 50°C for 19%
423 of points, despite water heater and principal return loop temperatures meeting
424 recommendations (Fig. 4c).
425
426 Additional temperature monitoring using surface thermocouples on subordinate flow
427 and return pipes were conducted on system 5 (Fig. S3). The ongoing temperature
428 monitoring in subordinate flow and return loops in addition to the principal flow and
429 return loop provided helpful information to identify local issues. For instance, broken
430 valves in a shower faucet resulted in cold water entering the hot water feed pipe and
431 riser. Fixing the device increased the minimal temperature by an average 5°C in all 10
432 subordinate risers in this wing (Fig. S3, a-c). A second example was insufficient

recirculation causing a significant heat loss during night flow, which was corrected by the addition of a local pump on the subordinate return loop, after the furthest pair of risers (Fig. S3, d-g). These examples show the importance of characterizing local conditions and the potential of single faulty devices to influence temperature maintenance in large sections of hot water distribution systems (HWDS). Again, we conclude that relying on temperature maintenance in the principal return loop is not sufficient to identify such risk areas.

3.3. *Legionella* monitoring

Results of microbiological measurements for the five studied systems are presented in Table 1. Detection by qPCR was used in complement of culture detection as it has been shown to be efficient in monitoring changes in the bacterial numbers (Krojgaard et al. 2011, Lee et al. 2011). Culture positive samples for *Lp* were detected in systems 4 and 5 with 22 and 27% positivity respectively (detection limit = 50 CFU/L; quantification limit = 250 CFU/L). Culture positive samples results were low, with only one count above quantification limit at 600 CFU/L, located at a tap in system 5. Positivity increased above 80% for both systems when measured by qPCR and remained below detection limit for systems 1-3, except for one sample in system 2 (Table 1, Table S2). Systems in which water temperature was kept consistently above 60°C coming out of the water heater and maintained above 55°C across the network were below detection limit for *Legionella* by culture or qPCR. Such results strongly suggest that satisfactory management of temperature at control points in the studied systems resulted in lower

455 prevalence. However, these results represent a water quality snapshot at a point in time
456 and are not necessarily representative of microbial quality over time or at other
457 locations in the HWDS. Several factors affecting *Lp* densities at a given point have been
458 identified including intrinsic biological system heterogeneity, culturability, prior
459 stagnation and sample volume. Napoli et al. showed variation of ≤ 20% concentrations
460 of CFU/ml from one day to the next within a ward during repeated sampling over five
461 consecutive days across eight units within a hospital (Napoli et al. 2009). In the present
462 study, confirmation sampling was conducted in two of the five HWDSs to investigate the
463 temporal variability. Fig. 5a shows results from repeated sampling conducted at three
464 control points (water heater outlet, principal return loop and a point-of-use) in systems
465 3 & 4. All samples were negative in qPCR and culture for system 1, whereas samples
466 from system 4 were consistently positive in qPCR and to a lesser degree in culture (Fig.
467 5a). Mean levels of *Lp* detected in system 4 were not significantly different between the
468 3 control points ($p > 0.05$). These findings are in agreement with recent reports of
469 discrepancies between trends in *Lp* by qPCR and culture in suboptimal conditions for
470 inactivation of viable but not culturable (VBNC) cells (Krojgaard et al. 2011, Lee et al.
471 2011). Krojgaard et al. showed that qPCR levels can be used to verify the impact of
472 corrective actions such as thermal shock and demonstrated non-detects qPCR results as
473 a predictor of low risk.
474
475 Another factor that may influence levels of *Legionella* in water is the duration of
476 stagnation prior to sampling. Recent evidence reported an increase in bacterial

concentrations after various stagnation times (overnight to 14 days) in the cold water distribution system of a large building (Lautenschlager et al. 2010, Lipphaus et al. 2014). A steady increase was observed in the first 12 hours of stagnation whereas longer stagnation time did not lead to further increase (Lautenschlager et al. 2010). In the present study, hot water was sampled from two taps at different stagnation times and *Lp* concentration was evalutated by qPCR (Fig. 5b). The taps were not found to be statistically different when comparing mean results and no correlation was established between the mean *Lp* concentration and the stagnation time. However, the stagnation times were longer than 12 hours, except for the 1h stagnation and samples were taken from the hot water systems. To our knowledge, there is no reported data on the impact of stagnation on bacterial concentrations in hot water. These results suggest that *Lp* concentrations in the first liter of hot water at the tap may not be affected by stagnation time.

The volume of sample determines the source of the water within the HWDS. *Lp* monitoring can be performed to assess the risk associated with 1) the water heater and primary distribution network using flushed samples, and 2) the distal system, including the tap and its connection to the main distribution system, using samples collected without prior flushing. Cristina et al. (2014) reported that distal stagnation increased the number of positive sites from 2.63 % to 15.79% and mean concentration from 7 vs 637 vs CFU/L for *Lp* sg1. Such distal amplification was not as clearly observed by these authors for *Lp* sg2-14 with 40.79 % to 42.11% positive and mean concentration from

19,455 vs 26,746 CFU/L. Similar trends were observed for *Legionella* spp in HWDS taps with increased concentration from 45 CFU/L (23% positivity) after a 3 minute flush to 226 CFU/L (35% positivity) in the first liter (Serrano-Suarez et al. 2013).

Although post-flush samples provide insight into systemic hot water distribution system (HWDS) contamination, results from the first volume to flow are indicative of the acute concentrations to which patients may be more readily exposed. In the first volume to flow from the tap, water temperature will often be lower due to previous stagnation and disinfectant will be absent, favoring culturability of cells. On the other hand, higher copper concentration present after prolonged stagnation could impact culturability. Non-detection of *Lp* by culture at a given sampling point and time doesn't necessarily equate to absence of risk for the system.

Volume sampled, typically 1L or more for *Lp*, plays an important role in data interpretation, either for temperature measurements or microbiological detection where the detection limit of the method improves with the use of higher volume of samples. As illustrated on Fig. 1, sampling the first liter will collect water from the tap and connecting pipes, and might reach water from the subordinate return and flow pipes depending on the configuration. For example, 8 meters of a 13 mm diameter pipe are required to reach 1L. If a larger sample volume is required to do multiple analyses (i.e. culture and qPCR or simultaneous detection of other waterborne opportunistic

pathogens), it should be kept in mind that water will become less representative of the point-of-use.

3.4. Value of Temperature Control in *Lp* Risk Management

The implementation of a water safety plan is the recommended approach for preventive risk-management related to drinking water (WHO 2011) and temperature control is widely recognized as the first risk mitigation measure for *Legionella* control in hot water distribution systems (HWDS) (Table S1).

Maintaining sufficient temperatures at all critical points, including the subordinate return loops, and minimizing volumes of uncontrolled temperature in the terminal ends appear essential to a successful system wide thermal control of culturable and VBNC *Legionella*. Most studies report on the results of temperature control based on prevalence measured by culture-based detection methods. Although lower prevalence is generally observed after temperatures are increased, limited efficacies are often reported. An early study observed 50% reduction of tap positivity following an increase in temperature from 45 to 60°C at the water heater outlet, although an elevated number of taps located in patient rooms remained positive (Ezzeddine et al. 1989). Water temperature at the tap ranged between 30 and 56°C after a few minutes of flushing, demonstrating the system's inability to provide elevated temperatures in all areas. A similar reduction in % positive taps from 60-90% to 30-40% was reported in a hospital when water heater temperature was raised from 50 to 65°C, in that case

providing temperatures >50°C at most outlets (Blanc et al. 2005). Importantly, the remaining positive outlets were situated in an area with inadequate recirculation. A third field study documented a successful reduction of *Legionella* positive taps from 100% to a mean value of 12% maintained over 10 years following the hot water temperature increase from 45 to 65°C (Darelid et al. 2002). This temperature regimen was implemented following an outbreak and resulted in water temperatures between 56 and 61°C at the tap after 5 minutes flushing. Recent field studies support the importance of maintaining elevated temperatures at distal locations (estimated by the temperature after 1 minute of flushing), with 4–11% of positive at T≥55°C vs 14-82% for T<55°C (Arvand et al. 2011, Bargellini et al. 2011, Hruba 2009). Those observations show that the efficiency of thermal inactivation in complex recirculated full scale HWDS is enhanced when temperature exposure is sufficient in all areas of the HWDS. However, significant distal amplification of *Legionella* can occur as evidenced by long term full scale sampling results (Cristina et al. 2014, Serrano-Suarez et al. 2013) and a number of taps may remain positive for *Legionella*.

The limitations of thermal control in HWDS raise questions on the validity of the existing threshold temperatures of 50-60^{o}C. Pioneer work evidenced the consistent susceptibility of 40 *Lp* isolates to temperature, with 1 log reduction achieved in 2.3-5 min at 60°C and 8 log reduction after 25 min as estimated by culturability (Stout et al. 1986). Recent findings show that elevated temperatures between 55 and 70°C will produce VBNC cells that cannot be detected by culture methods. Laboratory studies

564 conducted on HWDS samples confirm the suppression of culturability at T≥55°C as
565 evidenced by the presence of *Lp* when measured by qPCR and viable qPCR (Lee et al.
566 2011, Mansi et al. 2014). Despite a rapid loss of culturability at temperatures >55°C,
567 some *Lp* strains can resist in the VBNC state for periods of 30-60 minutes at
568 temperatures between 55 and 70°C (Allegra et al. 2008, Allegra et al. 2011, Epalle et al.
569 2014). Furthermore, the development of heat resistant *Lp* strains was observed over
570 time for groups of strains isolated in hospital water systems submitted to periodic
571 extreme temperature (24h @ 65°C a few times a year), while no such resistance was
572 observed for strains isolated from the system where heat shock treatments (70°C 30
573 minutes) were sparingly applied. Finally, the efficacy of thermal disinfection on biofilm,
574 the main reservoir of *Lp* in HWDS (Buse et al. 2014), is at best scarce and reports limited
575 and non-lasting efficacy of 70^{o}C for 2 hours on culturable *Legionella* spp (Saby et al.
576 2005). These findings stress that high temperature regimen provide *Lp* control not *Lp*
577 eradication and the importance of maintaining a constant temperature regimen
578 throughout the system to provide adequate contact time and avoid growth.
579
580 We propose a system wide risk classification to assess risk in a HWDS based on
581 published reports and our findings (Table 2). In addition to monitoring temperature at
582 critical control points, the evaluation criteria also include the percentage of time that
583 temperature is maintained at the hot water production unit or return loops. Indeed,
584 exposure to temperature should be considered instead of temperature alone, as
585 regulated for chemical disinfection (Concentration X Time concept). Subordinate return

586 loop temperatures are used to evaluate the system's heat loss within each sector of the
587 building. Temperature exposure in the subordinate flow and return loop is estimated
588 based on temperatures measured after 1 minute of flushing and serves to determine
589 risk in specific areas. When evaluating the five systems against the proposed risk
590 classification (Table 2), results from the characterization of the HWDS combined with
591 the temperature profiles at point-of-use were good predictors of areas at risk for *Lp*
592 detection (Table 1). In light of these findings and considering the presence of VBNC
593 *Legionella* at temperature ranging between 55-70°C (Epalle et al. 2014), the set points
594 proposed in existing regulations and guidelines and selected for the proposed risk
595 classification approach appear minimal and should be met at all times. The development
596 of heat resistant strains following periodic heat shock also supports the maintenance of
597 a steady thermal preventative inactivation regimen instead of relying on periodic
598 curative thermal shock (Allegra et al. 2011). The apparent limited success of HWDS in
599 large buildings may have been caused by inconsistent maintenance of sufficiently
600 elevated temperatures in all areas of the building because of inadequate recirculation
601 and/or low set-points.
602
603 Regulations and guidelines all recommend the identification of representative sampling
604 points for *Lp* sampling and temperature monitoring at designated control points.
605 However, the rationale for frequency and number of sites for temperature monitoring is
606 not evident and the limited number of proposed control points implies that the HWDS is
607 well balanced. Furthermore, there is little guidance for follow-up action to identify the

608 cause of temperature losses. To remediate this shortfall, a diagnostic flowchart for the
609 initial assessment of *Legionella* risk within an existing HWDS is proposed using
610 temperature measurements and profiles at the water heating unit, return loops and
611 critical points (Fig. 6). We propose a step approach starting from the principal return
612 and flow loop system that indicates the overall system risk level, then moving
613 progressively to the subordinate flow and return loops to identify large building areas or
614 sectors at risk, and finally to the tertiary terminal ends, to identify local issues with
615 defective faucets or showers. The diagnostic flowchart also proposes a staged response
616 in terms of corrective and preventative actions, including *Lp* monitoring. Critical control
617 points, defined as the water heater outlet, the principal return loop and representative
618 at risk points-of-use (not reaching control temperature, farthest from the water heater
619 or serving vulnerable patients) are prioritized for sectors or systems identified at risk by
620 the initial risk assessment (Fig. 6). This step approach can help direct efforts towards
621 high risk areas and optimize resource allocation, especially costly *Lp* monitoring.
622 Nevertheless, an ongoing *Lp* monitoring strategy and schedule should be put into place
623 through a water safety plan once initial assessment is completed and corrective
624 measures have been completed.
625
626 Although temperature control is a central element of risk mitigation, other factors
627 affecting the persistence of *Legionella* in HWDS should be considered in the water
628 safety plan such as: the susceptibility of environmental strains to heat inactivation; the
629 relative importance of terminal volumes not subject to recirculation; the sampling

protocol used for *Legionella* monitoring (first volume, flushing, etc.); the presence of biofilm & amoeba and the use of chemical disinfection. The limitations of traditional culture-based methods to detect the presence of *Lp* when in presence of environmental stressors should also be considered when determining a sampling strategy. Although culture is the gold standard and helpful to isolate strains, qPCR can be a valuable tool to monitor changes in a system. An increase in qPCR signal compared to a baseline is indicative of cell growth, even if there is no distinction between viable and dead cells. The relation between *Lp* culture and qPCR results is still not clear, but a recent study by Lee et al. showed qPCR results following the trends of culture in a hot water system, with exceptions for temperatures above 50°C and in the presence of additional disinfection (Lee et al. 2011). These exceptions may be attributed to the impact of temperature on culturability.

4. CONCLUSIONS

- A step approach combining temperature monitoring of the hot water distribution system (HWDS) main components and temperature profiling at points-of-use can be used to determine the susceptibility of overall hot water distribution system and specific areas of large buildings to *Legionella* proliferation. When multiple subordinate return loops are present, temperature should be monitored at each subordinate return loop prior to the principal return loop. Monitoring temperature representative points or even all points on

650 a rotating basis (e.g. 20%/year) is time consuming and yet insufficient for rapidly
651 detecting faulty equipment such as defective valves.

652 • The impact of faulty thermostatic devices extends far beyond the terminal
653 connecting piping and can affect large areas of buildings, placing significant
654 volumes of hot water at risk. Faulty return valves should be rapidly identified and
655 repaired or replaced. A change observed in results from continuous temperature
656 monitoring of the subordinate return loop can provide useful information to
657 identify the occurrence of a faulty device.

658 • Temperature monitoring will help understand the hydraulics, quantify the
659 thermal losses of the recirculating system and identify the distribution columns
660 that need balancing. A systematic diagnostic is necessary to identify areas most
661 at risk in hydraulically unbalanced HWDS or in older buildings where original
662 plans and drawings may not be available or renovations and rearrangements
663 have occurred.

664 • Systems assessment and monitoring should also take into account area specific
665 hydraulic conditions within the building, including closed units, low usage and
666 configuration of the overall system.

667 • Temperature profiling should be performed at a large number of points
668 confirming the extent (volume) and nature (systemic or distal) of undesirable
669 temperatures in HWDS, guiding *Lp* monitoring decisions. The staged approach
670 based on inexpensive and easily implemented temperature profiling can

optimize resources and funds allocation by directing efforts towards high risk areas.

- Although necessary, *Lp* monitoring is costly and time-consuming, and should be targeted to enable decision making for infection control. Our staged approach can guide corrective system interventions and serve as a basis to justify preventive risk reduction actions and select sampling points for *Lp* monitoring.

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850

851 **Figure legends:**

852 Fig. 1: Hot water distribution system general schematic including temperature control
853 points. Three different types of vertical and horizontal distribution systems are
854 represented: ① recirculation before the last tap; ② recirculation connected after each
855 device; ③ recirculation connected after the last device.

856 Fig. 2: Examples of continuous temperature monitoring at water heater outlet for each
857 studied system (black line) and at the return loop for system 4 and 5 (blue line). No
858 continuous monitoring data was available for systems 1 to 3. Date format is MM/DD/YY.

859 Fig. 3: Heat loss during stagnation of hot water in 1.25 cm diameter copper pipes with
860 and without insulation at room temperature.

861 Fig. 4: Hot water temperature profiles at points-of-use as a function of volume for a)
862 systems 1, 2 and 3 grouped, n = 7; b) system 4, n = 7; c) system 5, n = 36. Mean
863 temperature at the hot water production unit outlet and at the return loop are shown
864 for each system.

865 Fig. 5: Variability of *L. pneumophila* concentration measured by qPCR (a) in system 4, for
866 repeat sampling events without prior stagnation (n = 5, Jan-Oct 2013) (b) in system 5,
867 after different water stagnation times for tap A (light gray) and tap B (dark gray) (n = 2,
868 Nov-Oct 2012).

869

870 Fig. 6: Diagnostic flowchart for the initial assessment of *Legionella* risk in an existing
871 HWDS.

872 Table 1: HWDS Systems characterization through control points temperature and
873 microbiological measurements
874 Table 2: Proposed risk classification based on temperature control points

875

876