

# Testing for *Legionella* in Healthcare Facilities: Evaluation of the Reproducibility of *Legionella* Test Results and the Impact of Time on Viability and Variability

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## ABSTRACT

**Background/Objective:** Environmental surveillance for *Legionella* has been shown to be an important tool in assessing the risk for healthcare-associated Legionnaires' disease. Both the concentration of *Legionella* recovered in water from plumbing fixtures and the extent of colonization (percent positivity) have been used to assess risk.

**Results:** *Legionella pneumophila* and reliability of these parameters has been evaluated, but questions remain. The objectives of this study were 1) determine variability in *Legionella* concentration and percent positivity with repeated testing of water outlets within a hospital building and 2) evaluate changes in viability of *Legionella* over a time period typical for specimen transport.

**Methods:** Hot water from 12 sinks in a hospital administrative and outpatient building were sampled twice a week for 6-7 weeks for a total of 155 samples. Specimens were cultured for *Legionella* immediately after collection (T=0) and then held at room temperature before retesting after 1, 24, and 48 hr for a total of 620 cultures. Other *Legionella* species were consistently recovered from all 12 sinks. No significant difference in *Legionella* concentration was seen comparing T=0 vs. 1 and 24hr (p>0.05). After 48hr, recovery was slightly lower than T=0 (0.175 log; p<0.05), but this difference was less than day-to-day variability. The percentage of sites positive did not change throughout the study. The concentration of *Legionella* recovered varied over the study period. The average difference between the minimum and maximum for the 12 sinks over the study period was 0.96 log, with 0.58 – 1.34 log differences between the minimum and maximum. Day to day variability in concentration was higher than the difference between T=0 and 48hr hold times.

**Conclusions:** *Legionella* concentrations were stable during the typical time needed to transport a water sample for *Legionella* culture. Percent positivity did not change, however caution should be used in assessing risk for Legionnaires' disease based upon the concentration of *Legionella* recovered from a water fixture. Approximately 1 log variability in recovery was seen when testing the same fixtures repeatedly over time.

## METHODS

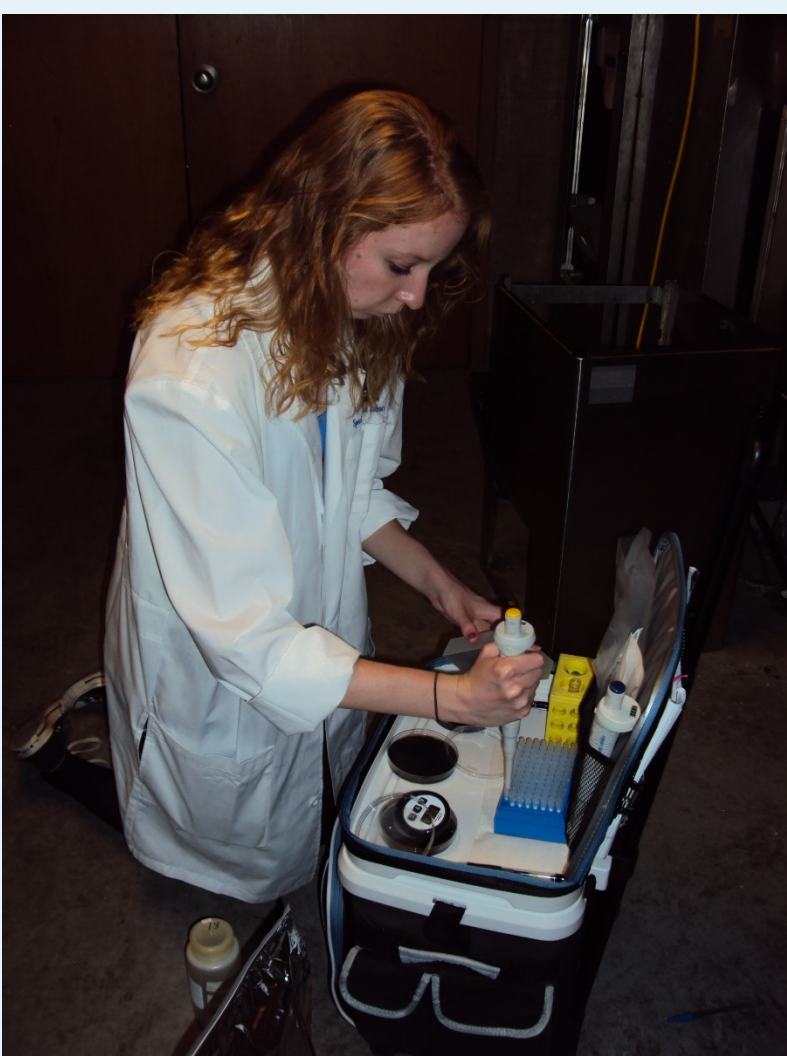


Figure 1. Processing water samples at T=0

Culture for *Legionella* was performed on 155 water samples as previously described (1, 2). Hot water samples (500 mL) were collected from 12 sites in a hospital office building 2 times a week for 6.5 weeks. On site, immediately after collection (T=0), the samples were directly to CCVC and DGVP (100 µL per plate) and spread. Upon returning to laboratory with all samples, T=0 plates were incubated at 36.5°C and T=1h plating was initiated. Samples were plated directly and after filter concentration to BCYE and DGVP. T=1h plates were incubated as above; the 12 bottles of samples were then left at room temperature.

A third plating was performed at 24 h and a fourth at 48 h. All sets were incubated for up to 7 days and examined for *Legionella pneumophila*

## RESULTS

**Viability:** No significant reduction in viability/recovery (log CFU/mL) of *Legionella pneumophila* was observed when samples were cultured within the time recommended from collection to processing, i.e. 48hr of collection (Figure 2, Tables 1 and 3). By 48hr, a decrease in mean recovery of 0.18 log CFU/mL was observed (Table 1, 3).

**Variability:** Average difference between min. and max. CFU/mL over the 13 sample days was 0.96 logs (range 0.58 – 1.34 log). There was no significant difference in standard deviation between T=0 and T=48 for 12 sinks over 13 sample days.

Table 1. Effect of time on recovery of *L. pneumophila* from water samples

Site	Log Mean CFU/ml (SD) at various holding times*			
	T=0 hr	T=1 hr	T=24 hr	T=48 hr
1. 3501 Utility Sink	2.22 (0.36)	2.14 (0.36)	2.00 (0.41)	2.07 (0.41)
2. 4519 Sink 2	2.05 (0.23)	1.94 (0.17)	1.91 (0.17)	1.62 (0.30)
3. 4519 Sink 4	1.98 (0.33)	2.05 (0.17)	1.94 (0.13)	1.86 (0.17)
4. 4510 Sink 1	2.35 (0.37)	2.29 (0.37)	2.27 (0.36)	2.14 (0.41)
5. 4510 Sink 3	2.36 (0.30)	2.40 (0.42)	2.30 (0.29)	2.14 (0.23)
6. 5515 Sink 1	2.04 (0.20)	2.09 (0.23)	2.04 (0.26)	1.88 (0.35)
7. 5515 Sink 3	2.15 (0.25)	2.14 (0.31)	2.13 (0.32)	2.08 (0.34)
8. 6501 Right Sink	1.85 (0.35)	1.94 (0.19)	1.96 (0.27)	1.68 (0.35)
9. 6507 B Right Sink	1.99 (0.22)	2.01 (0.28)	1.99 (0.29)	1.83 (0.34)
10. 10543 Right Sink	2.33 (0.25)	2.33 (0.20)	2.26 (0.27)	2.16 (0.31)
11. 10544 Sink	2.33 (0.18)	2.34 (0.16)	2.32 (0.25)	2.23 (0.20)
12. 11513 Sink	2.14 (0.22)	2.15 (0.20)	2.12 (0.22)	1.97 (0.31)

\*Samples were collected from each location for 6.5 weeks.

Figure 2. Bar graph shows relative difference in culture results over holding time.

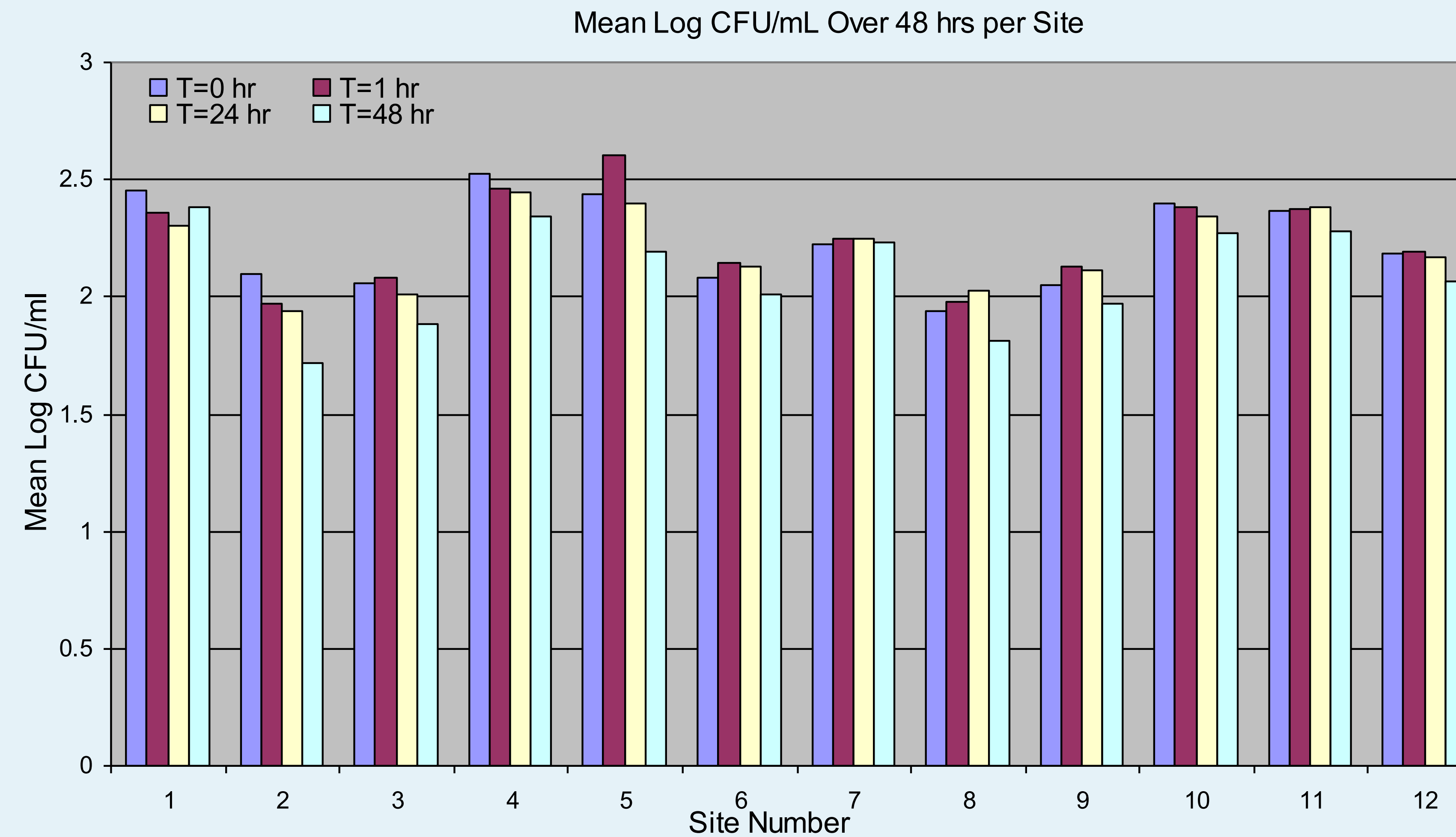


Table 2. No significant variation in recovery of *L. pneumophila* site-to-site or day-to-day.

Site	Results (CFU/ml) of biweekly sampling for 6 weeks of testing*					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1. 3501 Utility Sink	100/90	90/90	120/140	90/240	170/220	190/170
2. 4519 Sink 2	110/60	70/40	120/150	160/100	90/190	130/300
3. 4519 Sink 4	80/90	10/60	80/120	210/100	150/140	180/120
4. 4510 Sink 1	100/160	80/100	750/320	120/230	240/360	150/200
5. 4510 Sink 3	100/90	100/80	210/320	360/310	610/330	480/240
6. 5515 Sink 1	170/60	90/80	140/220	100/60	80/80	120/140
7. 5515 Sink 3	170/60	70/100	150/120	220/80	100/180	230/220
8. 6501 Right Sink	90/10	70/70	100/70	20/40	130/110	180/120
9. 6507 B Right Sink	90/50	70/120	70/100	90/60	90/100	90/140
10. 10543 Right Sink	280/310	100/350	90/220	200/410	170/150	120/200
11. 10544 Sink	240/320	140/310	150/170	120/160	260/200	190/210
12. 11513 Sink	110/60	220/100	210/210	80/60	160/160	180/150

\*Data shown are the T=0 results of CFU/mL on direct DGVP plates.

## RESULTS

Table 3. Mean recovery of *L. pneumophila* from 12 sink faucets sampled at 0, 1, 24 and 48 hours.

Time (hr)	Mean log cfu/mL	Mean difference	p-value
0	2.16	NA	NA
1	2.16	< 0.01	0.98 <sup>a</sup>
24	2.11	0.04	0.07 <sup>b</sup>
48	1.97	0.18	<0.05 <sup>c</sup>

<sup>a</sup> T0 vs T1; <sup>b</sup> T0 vs T24; <sup>c</sup> T0 vs T48

## CONCLUSIONS

*Legionella* has been shown to be stable and maintain viability in water over long periods of time (3). In our study, there was no significant increase or decrease in *Legionella* viability when cultured within the time recommended from collection to processing, i.e. 48hr of collection. Reduction in viability over time between sample collection and culture processing was not greater than the day-to-day variation, even at 48hr after collection.

Monitoring for *Legionella* from outlets (sinks) in complex building water systems is used to assess risk for disease (4). Recovery of *Legionella* from outlets was consistent both in log CFU/mL and proportion of sites positive in the building tested.

## REFERENCES

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Nothing to disclose.

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