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Investigation at a Veterans Affairs Medical Center (VAMC) of Spurious *Legionella* Environmental Testing Results and High Lab-to-Lab Variability among Four Commercial Laboratories

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Abstract

Background: The Dept. of Veterans Affairs (VA) requires quarterly water *Legionella* environmental testing (LET). The Minneapolis VAMC (MVAMC) began LET in 2008. All results were neg. until 11/2015, when a new (CDC ELITE-certified) LET lab (lab1) reported *Legionella spp*. (Lsp) in 12/40 (30%) MVAMC samples. Healthcare-associated legionellosis (HAL) and LET reliability were investigated.

Methods: Records of all 2015 MVAMC Lsp cases and potentially exposed patients were reviewed. In 1/2016, test and control water samples were sent to 4 contract LET labs. MVAMC water samples were collected from 5 purportedly Lsp-pos. sites. A sterilized-water neg. control and 3 pos. controls (10x dilutions of *L. pnueumophila* type 1 [Lp1] stock culture) were created. Each LET lab received 18 masked samples: 1 neg. control, 3 pos. controls, and 5 test samples, all in duplicate. Purported Lsp isolates underwent mass spectrometry (MALDI-TOF).

Results: During intensified LET (11/6/15-1/11/16), Lab1 ostensibly found Lsp in 77 (26%) of 296 MVAMC water samples. Mitigation and remediation was performed. No HAL cases were identified. The 4 LET labs' blinded test results (cfu/mL) were as shown (Table 1). In 2/2016 Lab3 tested all sites that Lab1 had reported as Lsp-pos., including areas not remediated; all were neg. for Lsp. By MALDI-TOF, all 18 purported MVAMC Lsp isolates from Lab1 were diverse non-Lsp environmental organisms. After learning of these results, Lab1 withdrew from its LET contract. CDC and VA experts were notified.

Conclusions: A (CDC-certified) LET lab provided spurious results, with enormous consequent costs to MVAMC. Lab-to-lab differences were found between the remaining 3 labs, raising concern about accuracy for both pos. and neg. LET results. Healthcare systems must be cautious in deciding when to perform LET and how to interpret the results.

Background

- *Legionella* are Gram-negative bacteria; live in aquatic environments and soil; thrive in biofilm
- More than 50 species of *Legionella*; half are thought to cause disease in humans
  - The most common species, *L. pneumophila*, includes at least 16 serogroups
  - *L. pneumophila* serogroup 1 (Lp1) is responsible for at least 70% of human illness
- *Legionella* lives inside cells of other microorganisms –> resistance to eradication by chemicals and heat
- Ideal growth temp 35-46°C (95-115°F); temps > 124 °F prevent biofilm formation
- Municipal use of monochloramine compared to free chlorine confers some reduced risk of human legionellosis
- VHA Directive 1061 – Feb 11, 2008, updated Aug 13, 2014
  - Healthcare facilities are recognized as areas of higher risk of *Legionella* transmission
  - 100% prevention of legionellosis is not possible; prevention efforts can reduce risk of exposure
  - Facilities should have a risk assessment policy
  - Monitor water temps; keep hot > 124°F (<110°F at point of use), cold < 67°F; monitor municipal water quality and biocide
  - Remove unused branch lines and dead-legs in facility fresh water system; flush regularly
  - Do clinical surveillance for legionellosis
  - Perform quarterly environmental *Legionella* water sampling: > 10 outlets each, hot and cold water, processed in a CDC ELITE certified lab. "Any amount of *L. pneumophila*...is considered a positive result."
  - Mitigation, further testing, and remediation is required if any positive results
- American Society of Heating and Air-Conditioning Engineers (ASHRAE) and American National Standards Institute (ANSI) Legionellosis and OSHA Technical Manual state that testing is indicated:
  - In the context of clinical legionellosis cases
  - In whirlpool/spas, if heterotrophic aerobic bacteria counts are high
- MVAMC performed routine environmental testing on Nov 6-8, 2015 as required by VHA
  - *Legionella* reportedly was detected in 12 of 40 water samples; 5 reportedly contained Lp1
  - This was the first positive water sample test for *Legionella* at MVAMC since testing began in 2008
  - A new LET contractor (Lab 1) had performed this water testing

Legionella pneumophila bacteria. PHIL CDC. http://phil.cdc.gov/phil/details.asp?pid=11150

Methods

- An intensive search for healthcare-associated legionellosis was undertaken
  - Medical records were reviewed symptoms of legionellosis and possible healthcare exposures for:
    - All patients with a positive *Legionella* test in the past 11 months
    - All patients who since Sept 8, 2015 (time since last negative testing) occupied a room with a purported positive water sample for *Legionella*
  - All providers were notified to test for *Legionella* in patients with appropriate symptoms
- Repeat Testing – 2 LET labs; Lab 1 and previous contractor
  - The two labs collected samples on different days, using their own collection techniques
  - Lab 1 had new positives. All results from previous contractor were negative.
- Repeat Testing – 4 LET labs; control all possible aspects of collection; compare inter- and intra-lab reproducibility
  - The 4 labs: current contractor (Lab 1); previous contractor and 2 additional CDC ELITE certified labs with long track records (Labs 2-4).
  - MVAMC staff collected 2 L of water from each of 5 purportedly *Legionella*-positive water sources that had not been remediated; 2 aliquots (A and B) per sample were prepared for each LET lab (Table 1).
  - 3 positive controls (10, 100, and 1000 cfu/mL of Lp1) were created using McFarland densitometry from laboratory stock Lp1 and diluted in sterilized water
  - Negative control: sterile water
  - All specimens and controls (250mL each) were aliquoted in a laminar flow hood (in sequence: negative controls -> test samples -> positive controls), then sent in duplicate to each lab
  - All laboratories were blinded to sample identify (pre-printed standardized labeling, standardized packaging, same sample size). Samples were all mailed on the same day (day of collection).
- Confirmatory testing of purported *Legionella* isolates from Lab 1
  - Primary testing at MVAMC using mass spectrometry (MALDI-TOF) and routine biochemical identification

Table 1. Results of 4 Lab Comparative *Legionella* Testing

Sample Type	Aliquot	Lab1 (cfu/mL)	Lab2 (cfu/mL)	Lab3 (cfu/mL)	Lab4 (cfu/mL)
Neg. control	A	*	*	*	*
	B	*	22.8 Lp1	*	*
Pos. Control 10 cfu/mL Lp1	A	*	*	2.8 Lp1	225 Lp1
	B	*	0.4 Lp1	2.8 Lp1	500 Lp1
Pos. Control 100 cfu/mL Lp1	A	1.0 Lp2-15; 35 Lsp	3.2 Lp1	28.2 Lp1	300 Lp1
	B	0.4 Lsp	1.2 Lp1	10.8 Lp1	1050 Lp1
Pos. Control 1000 cfu/mL Lp1	A	1.0 Lsp	24 Lp1	40.8 Lp1	825 Lp1
	B	*	*	260 Lp1	575 Lp1
Test Sample Site 1	A	*	*	*	*
	B	*	*	*	*
Test Sample Site 2	A	*	*	*	*
	B	*	*	*	*
Test Sample Site 3	A	*	*	*	*
	B	*	*	*	*
Test Sample Site 4	A	150 Lsp	*	*	*
	B	20 Lp2-15	*	*	*
Test Sample Site 5	A	*	*	*	*
	B	*	*	*	100 Lp1

"A" and "B" for each sample = duplicate specimens of the same type; "*" = negative test result; cfu/mL = colony-forming units per milliliter; Lp1 = *Legionella pneumophila* Type 1; Lp2-15 = *Legionella pneumophila* Types 2-15; Lsp =*Legionella* species, not *pneumophila*

Result accurate and reproducible
Result accurate, not reproducible
No difference in concertation detected across controls
Result neither accurate nor reproducible

Table 2. Confirmatory Identification of Purported Lab1 *Legionella* Isolates

	Lab1	MVAMC Laboratory			
Isolate Number	Lab 1 Identification	MALDI –TOF In vitro Diagnostic Database	MALDI –TOF In vitro Diagnostic Database Confidence value	MALDI –TOF Research Use Only Database	Select Gram and AFB Stain
L. pneumophila ATCC 33152	NA	*L. pneumophila*	99.9	*L. pneumophila*	
4-2	Lp1	No ID		*Mycobacterium mucogenicum*	AFB pos
4-4	Lsp	No ID		*Mycobacterium mucogenicum*	AFB pos
4-5	Lp2-15	No ID		*Mycobacterium mucogenicum*	AFB pos
5-1	Lsp	No ID		No ID	Gram neg coccobacillus
10	Lsp	*Leuconstoc mesenteroides*	99.9	*Leuconstoc pseudomesenteroides*	Gram pos cocci
20-4	Lp1	*Bergeyella zoohelcum*	98.1	*Sphingobacterium spiritivorum* vs *Chryseobacterium*	
20-7	Lp2-15	*Stenotrophomonas maltophilia*	99.9	*Stenotrophomonas maltophilia*	
23-1	Lsp	*Delftia acidovorans*	99.9	*Delftia acidovorans*	
23-2	Lsp	*Delftia acidovorans*	99.9	*Delftia acidovorans*	
24	Lsp	*Delftia acidovorans*	99.9	*Delftia acidovorans*	
25-2	Lsp	No ID		No ID	Gram neg rod
25-6	Lsp	*Staphylococcus warneri*	99.9	*Staphylococcus warneri*	Gram pos cocci
26	Lp1	*Chryseobacterium indologenes*	98.0	*Chryseobacterium* vs. *Nocardia*	
33	Lsp	*Brevundimonas diminuta*	99.9	*Brevundimonas diminuta*	
50-1	Lsp	No ID		No ID	Gram variable rod
61-1	Lsp	*Delftia acidovorans*	99.9	*Delftia acidovorans*	
61-2	Lsp	*Delftia acidovorans*	99.9	*Delftia acidovorans*	
68	Lsp	No ID		No ID	Gram pos rod

Lp1 = *Legionella pneumophila* Type 1; Lp2-15 = *Legionella pneumophila* Types 2-15; Lsp = *Legionella* species, not *pneumophila*

Results

1. **Concerns arose regarding Lab 1's technical practices**
   - "Unused" collection bottles this lab provided appeared to be reused (Fig 1.)
2. **Clinical surveillance identified no healthcare-associated legionellosis**
   - 2 cases of legionellosis were identified retrospectively; both had symptom onset prior to hospital admission
   - 0 of 292 patients who were in a room with a positive water sample for *Legionella* had symptoms consistent with legionellosis
   - No new cases of legionellosis were identified despite increased clinician awareness and testing.
3. **Results of repeat testing found variable results across all labs (Table 1)**
   - Only one lab (Lab3) had accurate and reproducible results across all 9 samples tested in duplicate
   - Lab 4 reported Lp1 in a test sample, but the paired aliquot was neg and no other labs reported this.
   - Lab 2 reported Lp1 in a neg control and a neg result in the 1,000 CFU/mL pos control, suggesting a labeling or data management error. It also failed to reproduce a pos. control result (10 CFU/mL).
   - Lab 1, the new LET lab, performed the worst. Pos control results were totally inaccurate (including reported non-Lp *Legionella* species). In 3 instances, results were irreproducible across duplicate aliquots.
4. **Confirmatory testing of purported *Legionella* isolates from Lab 1 found no *Legionella* (Table 2.)**
   - All 18 purported *Legionella* isolates from Lab 1 were confirmed as non-*Legionella*
   - The isolates were identified as representing instead diverse environmental organisms
5. **After Lab 1 was presented with these results it elected to terminate its water testing contract with MVAMC.** CDC and VA experts were notified of the testing issues identified.
6. **Subsequent testing by Lab 3 found no additional *Legionella*-positive water sample results,** including from areas where extensive remediation had taken place (which was costly and damaging to the plumbing) and areas that had not been remediated but were purportedly positive for *Legionella* per Lab 1

Figure 1.

Conclusions

- A CDC ELITE certified lab provided spurious results, with enormous consequent costs to MVAMC.
- When asked, all labs refused to provide detailed standard operating procedures for their testing, as they considered these proprietary. **There is lack of transparency as to how testing is performed**
- Changes in contract language and oversight may have led to a less experienced LET lab being selected
- **Despite all 4 labs being CDC ELITE certified, their results varied substantially; only one lab had reliable results. This raises serious concerns regarding the accuracy of the pos and neg test results that LET labs provide.**
- Oversight of laboratories that perform environmental *Legionella* testing (ELITE certification) is not the same as oversight of clinical laboratories and testing methods (FDA, CLSI, other)
- **Inaccurate lab testing led too:**
  - Substantial cost to respond/investigate: personnel time, materials, additional testing
  - Long-term impact to plumbing where remediation was performed unnecessarily
  - Damage to the public perception of our VA, since the positive test results were reported in the press
- **It is unclear what constitutes a "clinically significant" amount of *Legionella* found within environmental water sample.** Is 0.1 cfu, 1 cfu, 10 cfu, 100 cfu, or some other amount "clinically significant"? As companies advertise lower rates of detection, are the same remediation actions warranted?
- Healthcare systems must be cautious in deciding when to perform *Legionella* testing and how to interpret the results.

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