Spartan™

# On-site 45 min Legionella Detection System:
## Scientific results

Mar 22, 2018

# Validation overview

- **Objectives**
  1. Assess correlation between Spartan qPCR and laboratory culture
  2. Compare accuracy of Spartan qPCR vs. laboratory qPCR

- **Categorization of test results**
  - <10 GU/mL  Negative
  - 10-100 GU/mL  Positive
  - 101-1,000 GU/mL  Positive
  - >1,000 GU/mL  Positive

# Study design

- **51 cooling towers tested for *Legionella pneumophila* for 12 weeks**
  - Spartan qPCR: every 1 week
  - Regular laboratory culture: every 4 weeks
  - Locations: Ottawa, Toronto, Montreal Canada
- **Spartan qPCR**
  - Water samples tested immediately on-site
  - Results in 45 minutes
  - Genomic Units/mL (GU/mL)
- **Laboratory culture**
  - Water samples shipped to laboratory (takes 1-3 days)
  - Results in 10-14 days
  - Colony Forming Units/mL (CFU/mL)

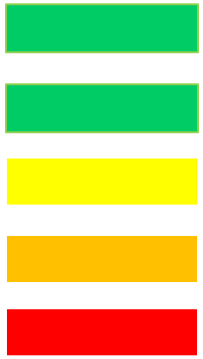
# Summary of results

# Summary of Spartan qPCR results

- **619 total Spartan qPCR tests**
  - 51 towers X 12 weeks X ~1 test/week

- **Spartan qPCR test results (Genomic Units/mL)**

| | | | |
|---|---|---|---|
| – No result: | 44 | (7.1%) | |
| – Undetectable: | 444 | (72%) | |
| – <10 GU/mL: | 52 | (8.4%) | |
| – 10-100 GU/mL: | 65 | (11%) | |
| – 100-1,000 GU/mL : | 13 | (2.1%) | |
| – >1,000 GU/mL: | 1 | (0.16%) | |



# Objective 1: Spartan qPCR vs. laboratory culture

# Spartan qPCR vs. laboratory culture

- **262 water samples tested with both Spartan qPCR and lab culture**
  - Spartan qPCR = Lab culture: 84% concordance
  - Spartan qPCR ≠ Lab culture: 16% discordance

- **Discordant results: why is lab culture under-diagnosing Legionella?**
  - Spartan positive and culture negative: 40/262 (15%)
  - Of the positive results, 40/64 (63%) were missed by culture

| | Spartan qPCR (>10 GU/mL) | Spartan qPCR (<10 GU/mL) |
|---|---|---|
| Lab culture (>10 CFU/mL) | 21 | 3 |
| Lab culture (<10 CFU/mL) | 40 | 198 |

# Hypothesis: shipping delay causes bacterial growth and degradation

# Summary of shipping delay experiments

| Relative Change Over Time | Delayed Laboratory Culture | Delayed Spartan qPCR | Delayed Laboratory qPCR |
|---|---|---|---|
| Degradation | 67% | 72% | 77% |
| Unchanged | 22% | 16% | 9% |
| Growth | 12% | 13% | 14% |

- **Conclusion: discordant results between Spartan qPCR and laboratory culture are explained by bacterial growth or degradation during shipping**

# Contrived shipping delay

**8 HVACs over 3 days spiked with live Legionella**

| Relative Change Over Time | Spiked HVACs, direct Spartan qPCR |
|---|---|
| Degradation (<2 fold) | 66% |
| No change | 23% |
| Growth (>2 fold) | 11% |

- **Conclusion: Not only are the bacteria dying the DNA is being rapidly degraded in 1-3 days**

# Objective 2: Spartan qPCR vs. laboratory qPCR

# Spartan qPCR vs. laboratory qPCR

- **Spartan qPCR vs. laboratory qPCR**
  - Spartan qPCR: water sample tested immediately on site, results in 45 minutes
  - Laboratory qPCR: 1-2 day shipping time delay, results in 1 day
- **45 water samples tested with both Spartan qPCR and lab qPCR**
  - Spartan qPCR = Lab qPCR: 36% concordance
  - Spartan qPCR ≠ Lab qPCR: 64% discordance
- **Discordant results: why is lab qPCR under-diagnosing Legionella?**
  - Reasons: bacterial degradation due to shipping delay

| | Spartan qPCR (> 10 GU/mL) | Spartan qPCR (<10 GU/mL) |
|---|---|---|
| Lab qPCR (> 10 GU/mL) | 6 | 3 |
| Lab qPCR (<10 GU/mL) | 26 | 10 |

# Laboratory qPCR vs. laboratory culture

- **Laboratory qPCR vs. laboratory culture**
  - Laboratory qPCR: 1-2 day shipping time delay, results in 1 day
  - Laboratory culture: 1-2 day shipping time delay, results in 10-14 days
- **43 water samples tested with both lab qPCR and lab culture**
  - Lab qPCR = Lab culture: 56% concordance
  - Lab qPCR ≠ Lab culture: 44% discordance
- **Discordant results: lab qPCR is under-diagnosing Legionella**

| | Lab qPCR (> 10 GU/mL) | Lab qPCR (< 10 GU/mL) |
|---|---|---|
| Lab culture (> 10 CFU/mL) | 3 | 12 |
| Lab culture (< 10 CFU/mL) | 7 | 21 |

# Case study: Cooling tower with Legionella levels >1,000 GU/mL

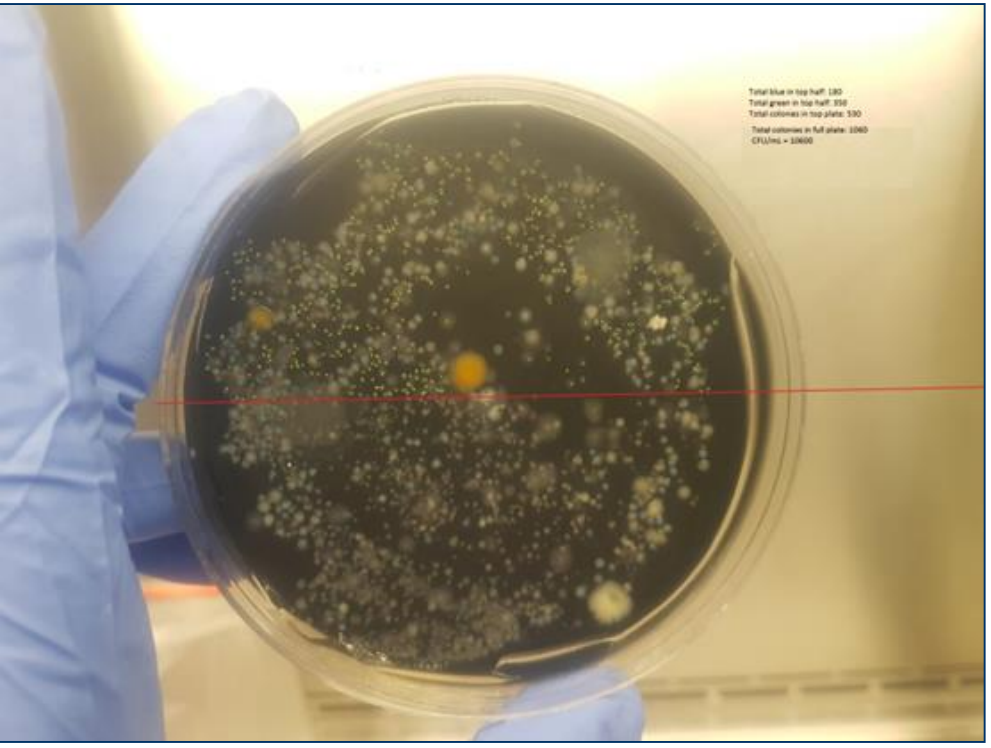
# Case study: culture underdiagnoses Legionella

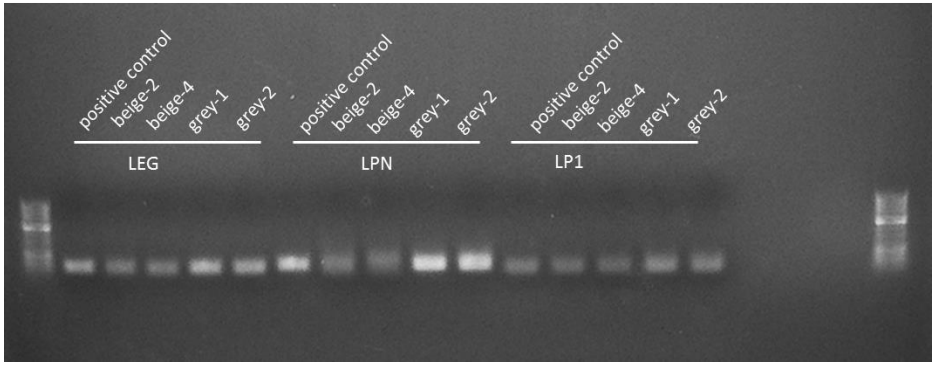
- **Cooling tower had levels of Legionella >1,000 GU/mL**
  - Lab qPCR and Lab #1 culture: under-diagnosed Legionella
  - Spartan qPCR and Lab #2 culture: diagnosed Legionella

| Test type | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 |
|---|---|---|---|---|---|
| Spartan qPCR (GU/mL) | 1,300† | 980 | 23 | 280 | 240 |
| Direct spike qPCR (GU/mL) | 3,100/3,300† | 1,100‡/1,700* | - | 730* | - |
| Lab qPCR (GU/mL) | <0.5‡, 8,100 | - | - | - | - |
| Lab #1 culture (CFU/mL) | 5† | - | <1 | - | - |
| Lab #2 culture (CFU/mL) | 960* | 320* | <1* | FedEx Lost | 140‡ |
| Spartan Culture (CFU/mL) | 11,000* | 2,000‡ | <4‡ | - | - |

† Time delay of 1 day
‡ Time delay of 2 days
* Time delay of 3 days

# Spartan qPCR: confirmed with direct plating






- **Direct plating**
  - HVAC water was directly plated without filter concentration or shipping
  - Colonies were counted manually
  - Legionella result: 11,000 CFU/mL
- **Confirmatory colony PCR**
  - 20 colonies were randomly selected
  - PCR performed for 3 targets: *Legionella* species, *L. pneumophila*, *L. pneumophila* serogroup 1
  - All 20 colonies were positive for all 3 targets

# Why is culture inconsistent: shipping delay + culture method

# Different culture methods give different results

- **19 Spartan qPCR positive samples sent for culture**
- **Samples sent in parallel to 2 different laboratories**
- **Lab #1 followed the ISO method and the Lab # 2 followed the CDC method**
  - Lab #1 was 37X higher on average
- **Question: Why the difference between laboratories?**

| Lab #1 - CDC method (CFU/mL) | Lab #2 - ISO method (CFU/mL) | Lab1/Lab2 Fold difference |
|---|---|---|
| 520 | 94 | 6 |
| 500 | 23 | 22 |
| 320 | 11 | 29 |
| 120 | 6 | 20 |
| 80 | 1 | 80 |
| 60 | <1 | 60 |
| 40 | <1 | 40 |
| 40 | 1 | 40 |
| 20 | 1 | 20 |
| <1 | 73 | 0.01 |
| <1 | 7 | 0.1 |
| <1 | 2 | 0.5 |
| <1 | 2 | 0.5 |
| <1 | 1 | 1 |
| <1 | <1 | 1 |
| <1 | <1 | 1 |
| <1 | <1 | 1 |
| <1 | <1 | 1 |
| <1 | <1 | 1 |

# Pre-treatments correlate with lower results

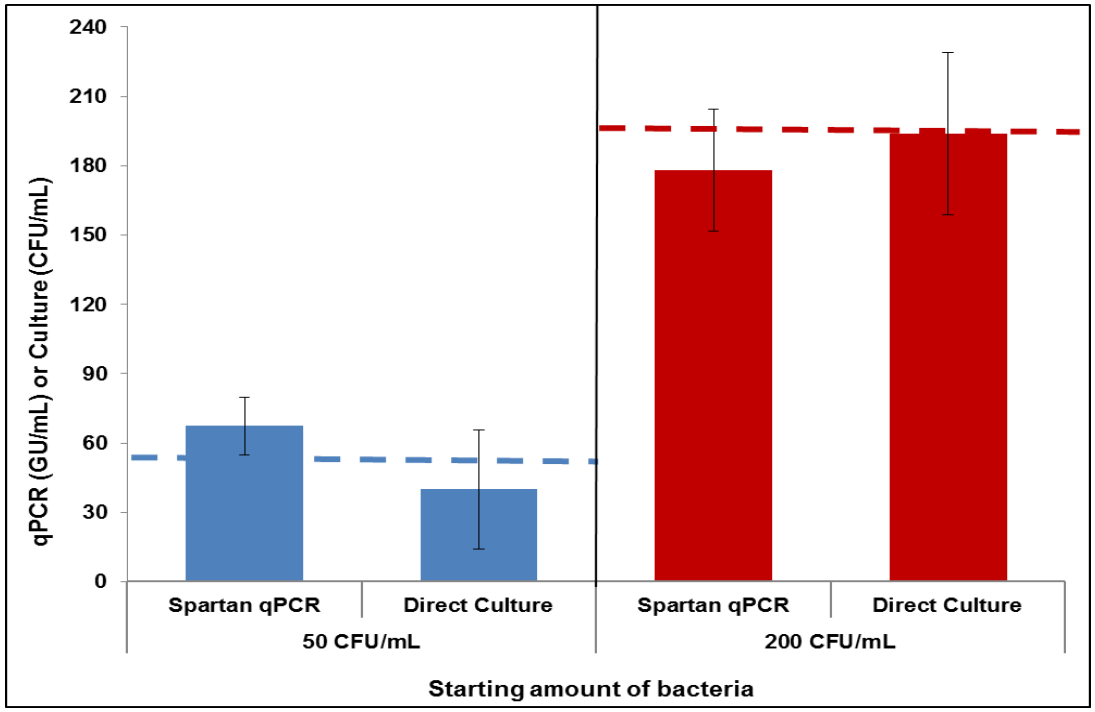
- Labs treat samples with acid or heat to remove other bacteria
- ISO methods use pretreatments more often than CDC methods
- Pretreatments frequently associated with lower levels of Legionella

- Conclusion: Culture methods under-report levels of live Legionella

| Lab Culture Result (CFU/mL) | Treatment |
|---|---|
| 960 | - |
| 520 | - |
| 500 | - |
| 320 | - |
| 120 | - |
| 94 | Acid |
| 88 | Heat |
| 80 | - |
| 73 | Heat |
| 60 | - |
| 40 | - |
| 40 | - |
| 40 | - |
| 23 | Acid |
| 20 | - |
| 11 | Heat |
| 9 | Heat |
| 7 | Acid |
| 7 | Heat |
| 6 | Acid |
| 5 | - |
| 5 | Heat |
| 3 | Heat |
| 2 | - |
| 2 | Acid |
| 2 | Heat |
| 1 | Acid |
| 1 | Acid |
| 1 | Acid |

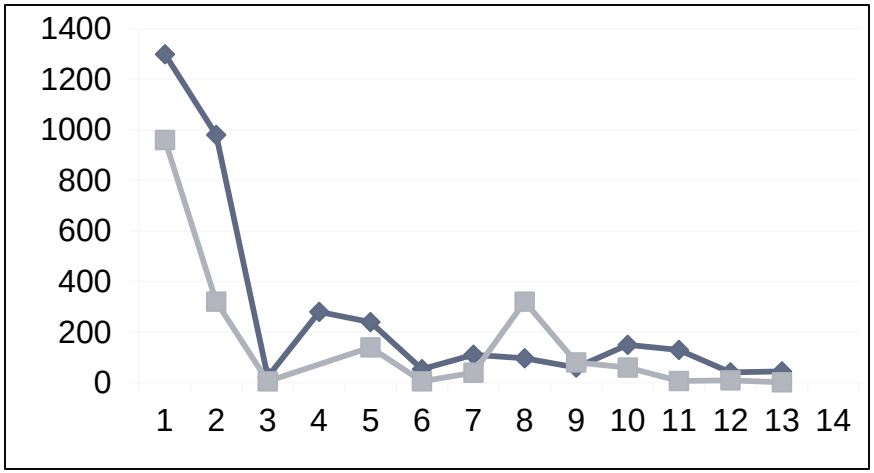
# Spartan qPCR and direct culture are equivalent

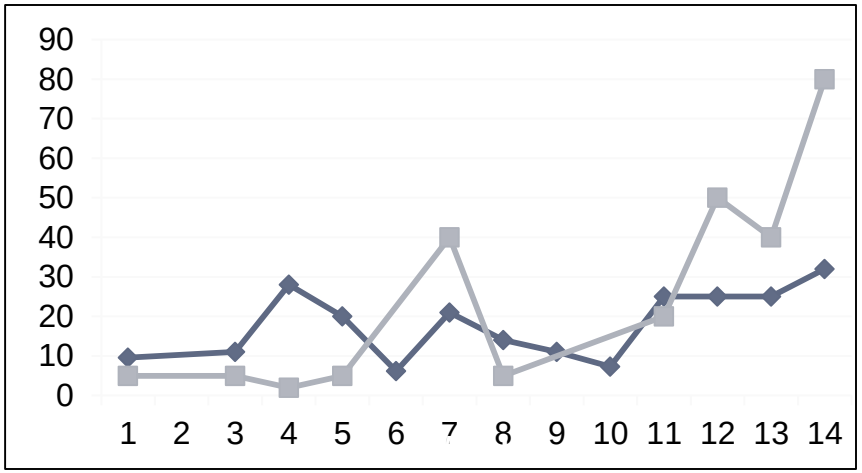
- **Spartan qPCR was directly compared to culture**
  - Samples were tested immediately (no shipping delay)
  - Samples had no pretreatments

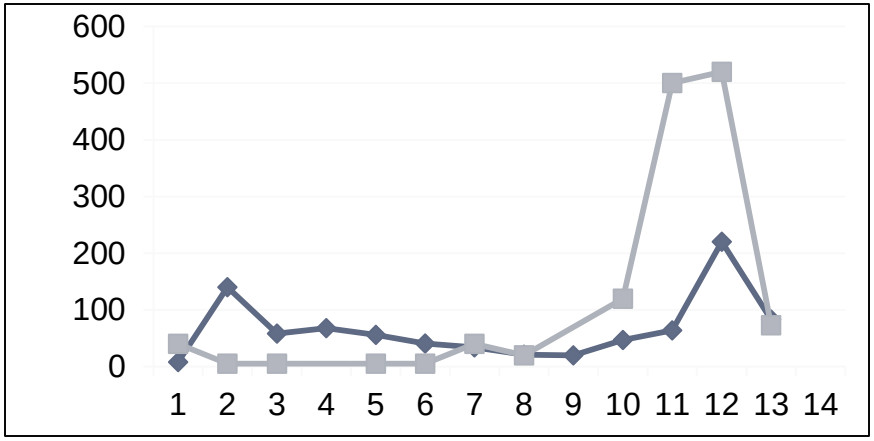


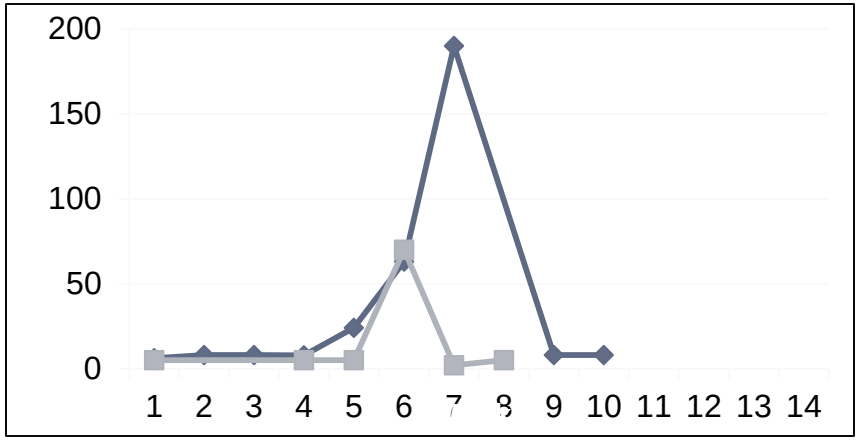

- **Conclusion: On-site Spartan qPCR is accurate**

# Spartan qPCR and culture correlated well



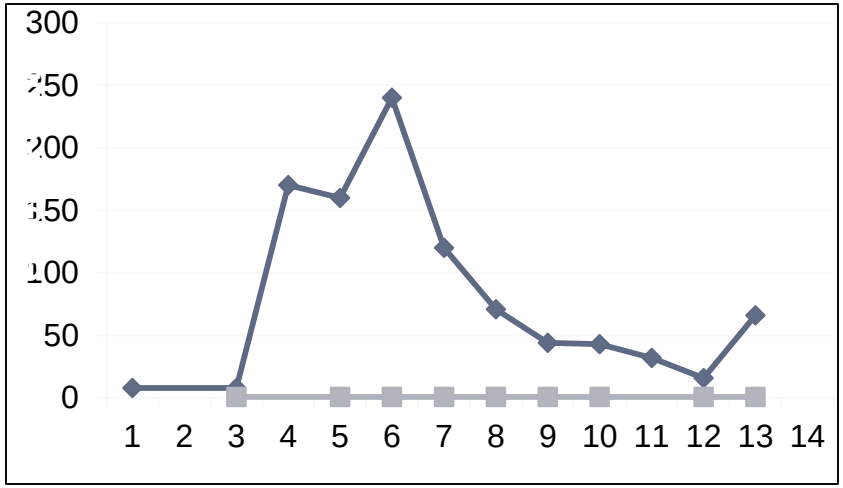



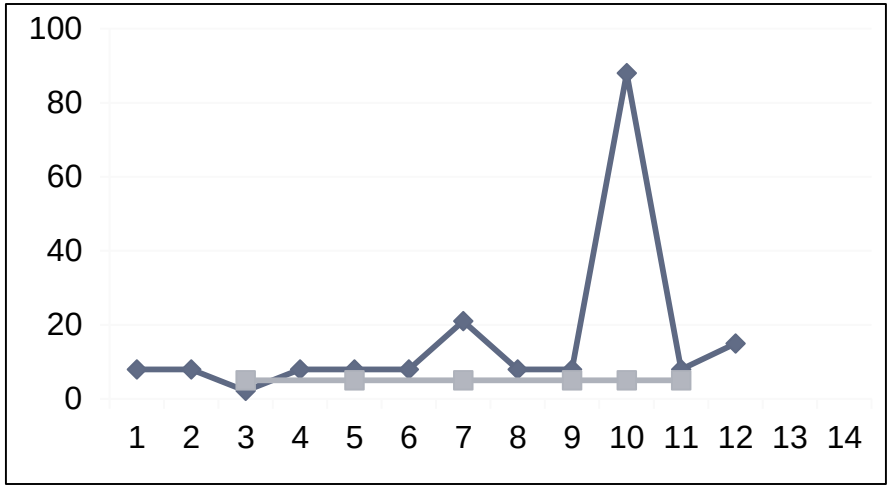



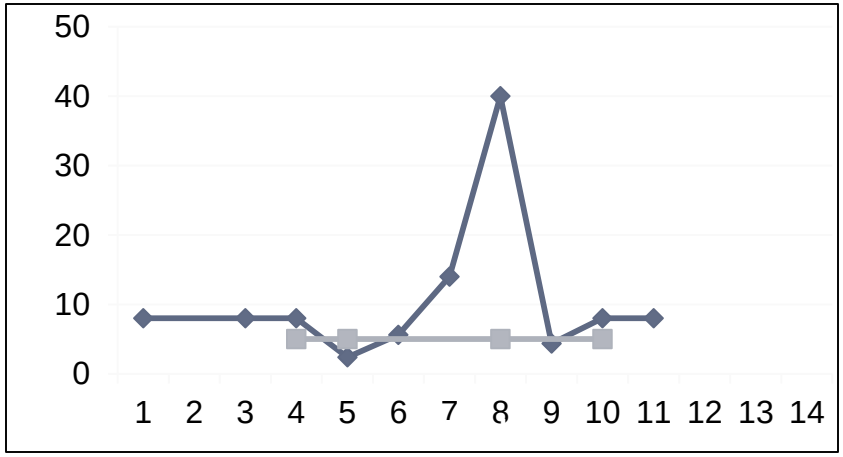

Lab culture

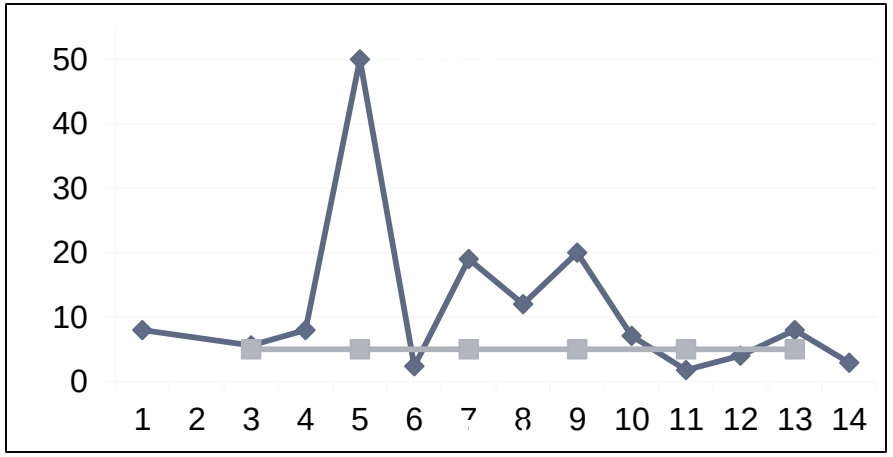
Spartan qPCR

# Spartan qPCR and culture correlated poorly




Lab culture

Spartan qPCR

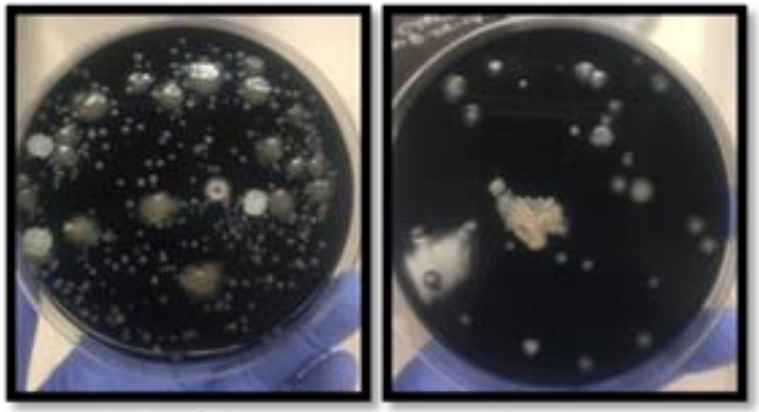
# Case study of poor correlation

| Sampling Date | Spartan qPCR GU/mL | Lab Culture CFU/mL |
|---|---|---|
| 15-Jan | 87 | - |
| 12-Jan | 36 | - |
| 11-Jan | 36 | - |
| 04-Jan | 98 | 5 |
| 03-Jan | 250 | - |
| 28-Dec | 120 | - |
| 28-Dec | 110 | - |
| 21-Dec | 63 | - |
| 14-Dec | 17 | <5 |
| 07-Dec | 76 | 5 |
| 29-Nov | 49 | <5 |
| 22-Nov | 40 | - |
| 16-Nov | 25 | - |
| 08-Nov | 24 | <5 |

# Live Legionella in the water, biofilms and sludge

| Source | Spartan qPCR (GU/mL) | Direct Culture (CFU/mL) |
|---|---|---|
| Sample point | 32 | 15 |
| Reservoir | 31 | 10 |
| Exit pipe | <LOD | <LOD |
| Biofilm-Reservoir | Not tested | 26 000* |
| Sludge-Reservoir | 1700 | 3000 |

* CFU/g as the biofilm was a solid



Biofilm Sludge

# Key findings

- Spartan qPCR results were falsely identified as negative by culture due to bacterial degradation during shipping and culture methods
- Spartan qPCR is equivalent to culture when shipping delay and culture pretreatments are removed
- Case study: one tower had Legionella >1,000 GU/mL that was missed by regularly-scheduled culture and dipslide testing
- On-site culture confirmed high amounts of Legionella in biofilms missed by laboratory culture
- Spartan qPCR is a robust means of monitoring Legionella levels

# Main recommendations

- Use on-site qPCR to monitor Legionella levels
- Set 4 action levels for qPCR testing:

| Level (GU/mL) | Action |
|---|---|
| <10 | Continue Operation |
| 10-100 | Review and Adjust O&M and Water Treatment Program |
| 101-1,000 | Clean and Disinfect System |
| >1,000 | Immediately Implement Measures that will Eliminate Water Dispersion by Aerosols |