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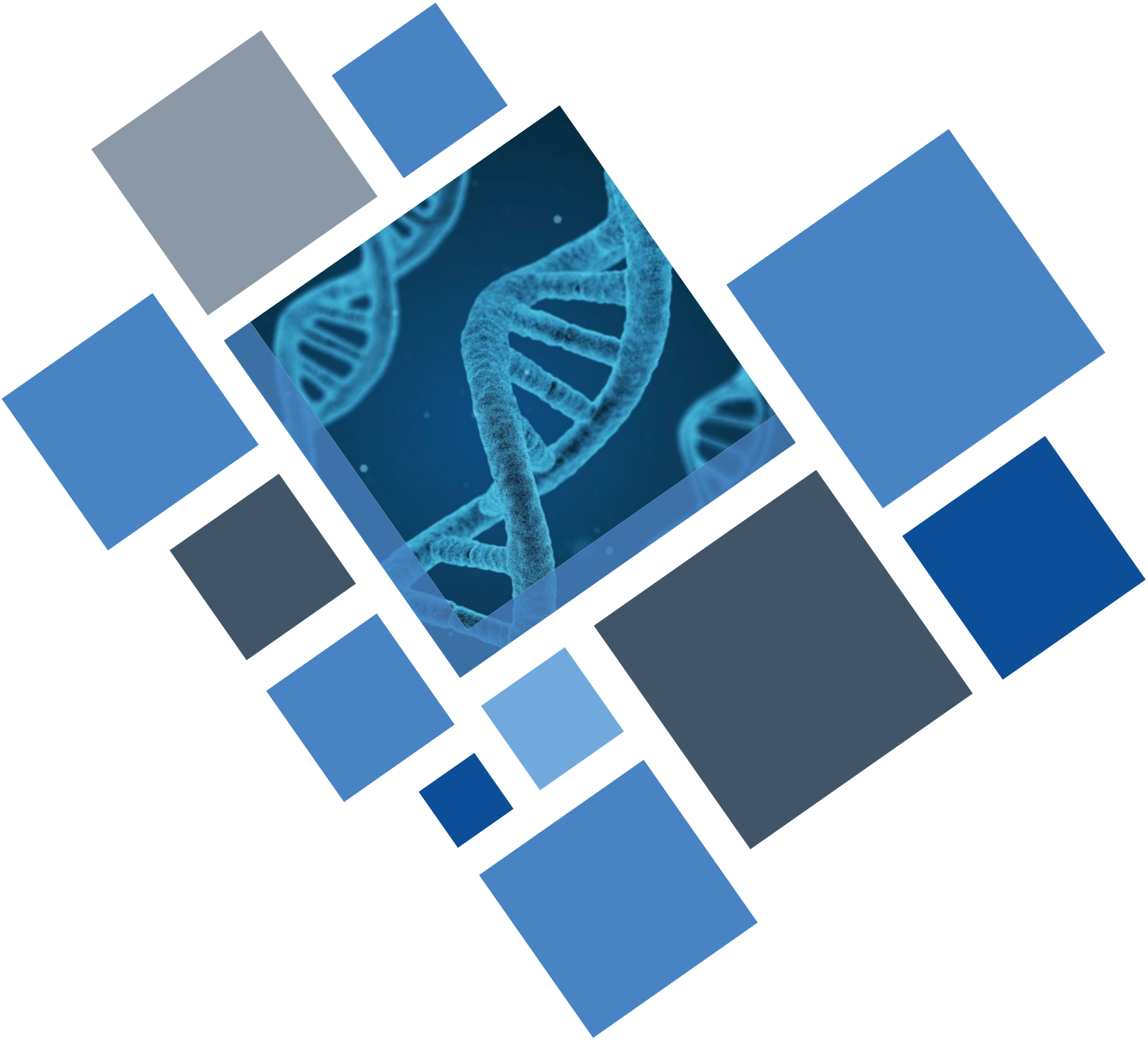
SCIENTIFIC BRIEFING :
ON-SITE LEGIONELLA DNA TESTING



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ON-SITE LEGIONELLA TESTING: SCIENTFIC BRIEFING

New award-winning Legionella testing technology detects water contamination in buildings

SUMMARY

- Legionnaires' disease is a common and potentially fatal pneumonia caused by inhaling water vapour contaminated by Legionella bacteria
- Cooling towers in buildings are frequently contaminated with Legionella
- Traditional Legionella testing methods are slow and inaccurate, and have proven ineffective at detecting Legionella contamination
- Spartan Bioscience has developed the first and only on-site Legionella test that is fast and accurate for detecting Legionella contamination before it grows out of control
    - The test is the 2018 winner of the HVAC industry's top innovation award for Indoor Air Quality
    - Customers include leading public health authorities such as the U.S. Centers for Disease Control & Prevention (CDC) and the world's largest real estate companies, healthcare organizations, hotels, data centers, and manufacturing facilities

SCIENTIFIC BACKGROUND

LEGIONNAIRES' DISEASE IS COMMON AND UNDERDIAGNOSED

- Legionnaires' is a severe pneumonia with a mortality rate ranging between 5-20% that is caused by breathing in water vapor contaminated with Legionella bacteria[1]
- High-risk groups include smokers, people over the age of 50, and those who are immunocompromised, but healthy people of all ages can be infected[2]
- Legionnaires' in the United States
    - Community-acquired pneumonia (CAP) affects 5.6 million patients annually in the US[3]
    - It is estimated that Legionnaires' accounts for 2–9% of cases of CAP[2]
        - i.e., 100K-500K cases per year, with a mortality rate of 5-20%
        - For comparison, asbestos-related cancer kills only 2,579 people per year in the US[4]

- According to the Centers for Disease Control & Prevention (CDC), 8,000–18,000 patients are hospitalized with Legionnaires' each year[5]
  - However, cases reported to the CDC "probably represent less than 5% of actual cases."[2]
  - The reason for the underreporting of cases is that pneumonia patients are not routinely tested for Legionnaires'. When they are, studies have found that 3.7% of non-hospitalized patients and 14% of hospitalized patients tested positive for Legionnaires'.[6]

- Legionnaires' in Canada
  - Public Health Ontario (PHO) confirmed 203 cases of Legionnaires' in the province in 2017[7]
  - But PHO estimates that the true number of cases is 7,574 per year in Ontario[8] [i.e., confirmed cases probably represent less than 5% of actual cases] [this number corresponds to approximately 20,000 cases across Canada]
    - For comparison, asbestos-related cancer kills only 467 people per year in Canada[9]
  - In Summer 2018, Humber River Hospital (North York, ON) conducted a clinical study in which hospitalized pneumonia patients were tested for Legionnaires'.10 of 32 patients, 9 tested positive for Legionnaires' (28%). 4 of the 9 patients were so sick that they required admission to the Intensive Care Unit (ICU). Based on these findings, discussions are underway to replicate this study with hospitals in the Ottawa-Gatineau region.

LEGIONELLA CONTAMINATION OF WATER SYSTEMS IN BUILDINGS IS COMMON

- Based on investigations of Legionella outbreaks, the CDC found that the most common sources of Legionella infection were:[11]
  - Potable water (e.g., showers): 56%
  - Cooling towers: 22%
  - Hot tubs: 7%
  - Industrial equipment: 4%
  - Decorative fountains: 4%
- Also, the CDC found that the most common outbreak settings were:
  - Hotels and resorts: 44%
  - Long-term care facilities: 19%
  - Hospitals: 15%
  - Senior living facilities: 7%
  - Workplaces: 7%
  - Community: 7%

- Legionella contamination of water sources in buildings is common:
    - In a study of healthcare facilities, 16% of cold-water sources and 6% of warm-water sources had *Legionella pneumophila* at concentrations >10 bacteria/mL[12]
    - The CDC collected water samples from cooling towers across the US and found *Legionella pneumophila* growing in 27% of towers[13]
    - In a study of hot-water showers in swimming pools, 40% of samples tested positive for *Legionella pneumophila*[14]

GROWTH OF LEGIONELLA BACTERIA

- There are more than 50 Legionella species, but *Legionella pneumophila* is the cause of 90%-95% or more of Legionnaires' cases[15,16]
- Legionnaires' disease outbreaks typically occur when *L. pneumophila* concentrations reach 1,000 bacteria/mL. However, lower concentrations may be dangerous for the young, elderly, and immunocompromised. For example, CDC guidelines recommend maintaining undetectable levels in hospitals with transplant units.[1,17]
- In water systems in buildings, the doubling time of *L. pneumophila* is typically between 22–72 hours, although the doubling time can be as few as 150 minutes, as reported in a case to investigators from the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE).[18,19] This means Legionella can reach outbreak levels of 1,000 bacteria/mL in as few as 7 days.
- *L. pneumophila* grows quickly at temperatures between 25°C-42°C, with an optimal growth temperature of 35°C.[15]

LEGIONELLA TESTING METHODS

Testing methods	Time to result	Detects live not dead bacteria?	Avoids false negatives from bacterial degradation during shipping?	Accuracy
Dipslides	24-48 hours	Yes	Yes	Poor
Lab culture	10-14 days	Yes	No	Good
Lab qPCR	2-7 days	No	No	Good
On-site qPCR	45 minutes	Yes	Yes	Excellent

DIPSLIDES

- Dipslides are performed on-site by growing bacteria from a water sample on a nutrient strip
    - Results are available in 24-48 hours

- Dipslides do not distinguish between Legionella and other bacterial species
- Dipslides have a real-world limit of detection of 10,000 bacteria/mL[20,21]
    - i.e., this poor sensitivity means that dipslides fail to detect almost all cases of Legionella contamination

LAB CULTURE

- Culture tests are performed by shipping a water sample to a lab where it is grown on a Petri dish
    - Results are available in 10-14 days because it takes this long for the Legionella bacteria to grow
- The Petri dish technology which underlies culture and dipslide tests was invented in 1887
- Culture testing has a real-world limit of detection of approximately 10 bacteria/mL even if concentrating a 250-mL or 1,000-mL sample[22,23]
- The CDC found that certified culture tests undercounted actual Legionella concentrations by 1.25 log (17-fold) and values differed between labs by 0.78 log (6-fold)[23]
    - On average, culture had a false negative rate of 11.5% [and this was with proficiency samples which are cleaner and have less inhibitors than real-world water samples]
- Australian public health scientists found that culture tests completely failed to detect Legionella in an outbreak investigation, but Legionella was accurately detected by qPCR tests[24]
- In a head-to-head study of lab culture vs. on-site qPCR for cooling towers, on-site qPCR detected Legionella bacteria above the acceptable limit of 10 bacteria/mL in 40% of cooling towers vs. only 15% of towers with lab culture[25]
    - A major reason for culture's poor performance was that bacteria degraded in 72% of water samples shipped to the lab
    - This caused culture to fail to detect a tower with contamination >1,000 bacteria/mL
    - 21% of cooling towers experienced rapid Legionella growth of 3X to 21X over 7 days
- Several jurisdictions have implemented mandatory culture testing of cooling towers, but this has failed to prevent ongoing disease outbreaks due to the poor performance of culture
    - e.g., During the summer of 2015, a Legionella-contaminated cooling tower caused an outbreak of Legionnaires' disease in New York City that killed 16 people and sickened 138 others.[26] In 2016, NYC required culture testing for all cooling towers. Despite this mandatory testing, the city continues to experience outbreaks. For example, an outbreak on the Upper East Side killed 1 person and sickened 6 more.[27] This was followed by an outbreak that sickened 14 people in the Flushing neighborhood of Queens.[28]

LAB QPCR

- qPCR tests utilize a Nobel-Prize-winning chemistry called quantitative Polymerase Chain Reaction (qPCR) to detect the DNA of organisms such as Legionella bacteria
    - In medical diagnostics, qPCR tests have replaced culture tests because qPCR is significantly faster and more accurate
- A scientific review of 28 studies involving 3,967 water samples found that qPCR was significantly more sensitive than culture at detecting Legionella, and culture consistently underreported Legionella levels[29]
- Leading organizations such as the CDC, New York State Department of Health (NYS DOH), and New York City Department of Health (NYC DOH) use qPCR tests for investigations of Legionnaires' outbreaks[30,31]
- qPCR tests may be performed on-site or by shipping a water sample to a laboratory
- Lab qPCR tests suffer from the following limitations:
    - They detect both live and dead bacteria, which means they can over-call the amount of pathogenic bacteria, thus leading to overtreatment of the water source[32]
    - In a study that compared on-site qPCR with lab qPCR, Legionella bacteria degraded in 77% of water samples shipped to the lab, which led to underestimates of the true amount of Legionella[25]

ON-SITE QPCR

- Spartan Bioscience has developed the first and only on-site Legionella qPCR test
    - It provides results in 45 minutes, in contrast to 10-14 days with culture testing
    - It provides results immediately on site, which means there is no bacterial degradation or false negative results from shipping water samples to a lab
    - It has patent-pending technology that detects live and not dead Legionella bacteria[25]
    - It is calibrated to culture so that 1 Genomic Unit (GU)/mL with Spartan's test is equivalent to 1 Colony Forming Unit (CFU)/mL with culture, which means that positive test results may be disinfected according to existing industry action levels
    - The test is validated according to the ISO 12869 standard for Legionella qPCR and has a limit of detection of 8 bacteria/mL[25]
        - Risk analysis research shows that a limit of detection of 10 bacteria/mL is effective at preventing Legionnaires' disease even for high-risk patients such as those undergoing organ transplant, chemotherapy, or dialysis[22]
- Spartan's on-site test is the winner of AHR Expo's 2018 Innovation Award for Indoor Air Quality (this is the HVAC industry's most prestigious innovation award)
- Customers of the test include expert Legionella organizations such as the CDC, New York State Department of Health (NYS DOH), and Virginia Tech (the team that solved the Flint, Michigan water crisis)

EXAMPLES OF LEGIONELLA TESTING STANDARDS AROUND THE WORLD

CANADA: PUBLIC SERVICES AND PROCUREMENT CANADA (PSPC) - MD 15161 STANDARD

- Cooling towers
    - Test weekly with dipslides and monthly with culture
    - Test with qPCR when starting up a tower and to confirm disinfection following a positive culture result
- Open water systems e.g., decorative fountains
    - Test weekly with dipslides and every 2 months with culture
    - Test with qPCR when starting up the water system and to confirm disinfection following a positive culture result
- Humidifiers
    - Test non-steam humidifiers monthly with dipslides and every 3 months with culture
    - Test steam humidifiers every 3 months with culture
    - Test with qPCR to confirm disinfection following a positive culture result
- Domestic hot water systems
    - Test monthly with dipslides (for systems connected to shower facilities and with a tank <50°C)
    - Test every 6 months with culture (for the most remote shower facility)
    - Test annually for all other domestic hot water systems
    - Test with qPCR to confirm disinfection following a positive culture result

UNITED STATES: CENTERS FOR MEDICARE & MEDICAID SERVICES (CMS) - QSO-17-30 DIRECTIVE

- As of July 6, 2018, all Hospitals, Critical Access Hospitals (CAHs) and Long-Term Care (LTC) "must have water management plans and documentation that, at a minimum, ensure each facility: Specifies testing protocols and acceptable ranges for control measures, and document the results of testing and corrective actions taken when control limits are not maintained."

NEW YORK CITY: LOCAL LAW 77 AND CHAPTER 8 RULES

- Cooling towers must be tested weekly with dipslides and every 90 days with culture

UNITED KINGDOM: HSG282 (2017)

- Spa pools and hot tubs should be tested quarterly

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