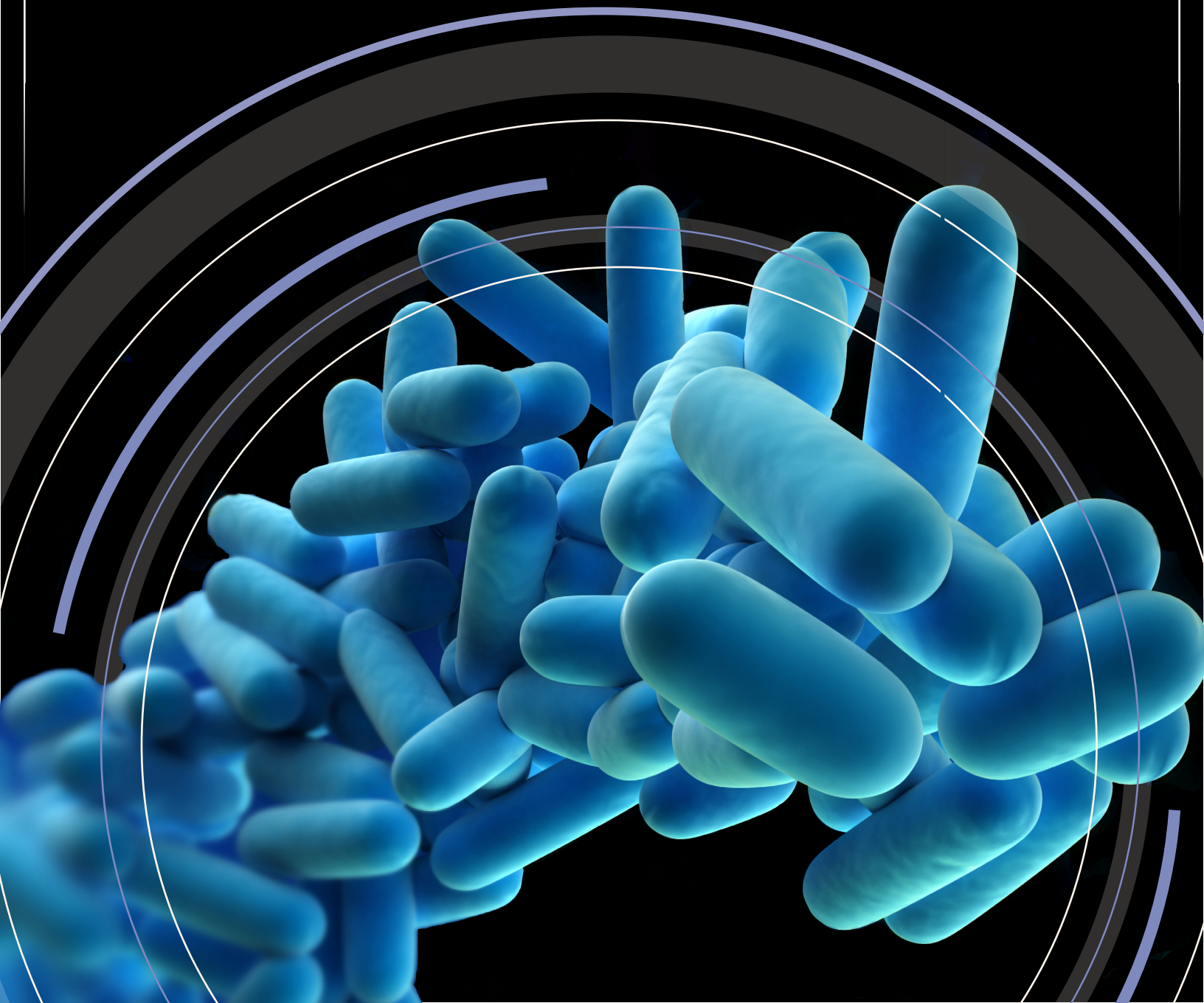




Built Environment Testing

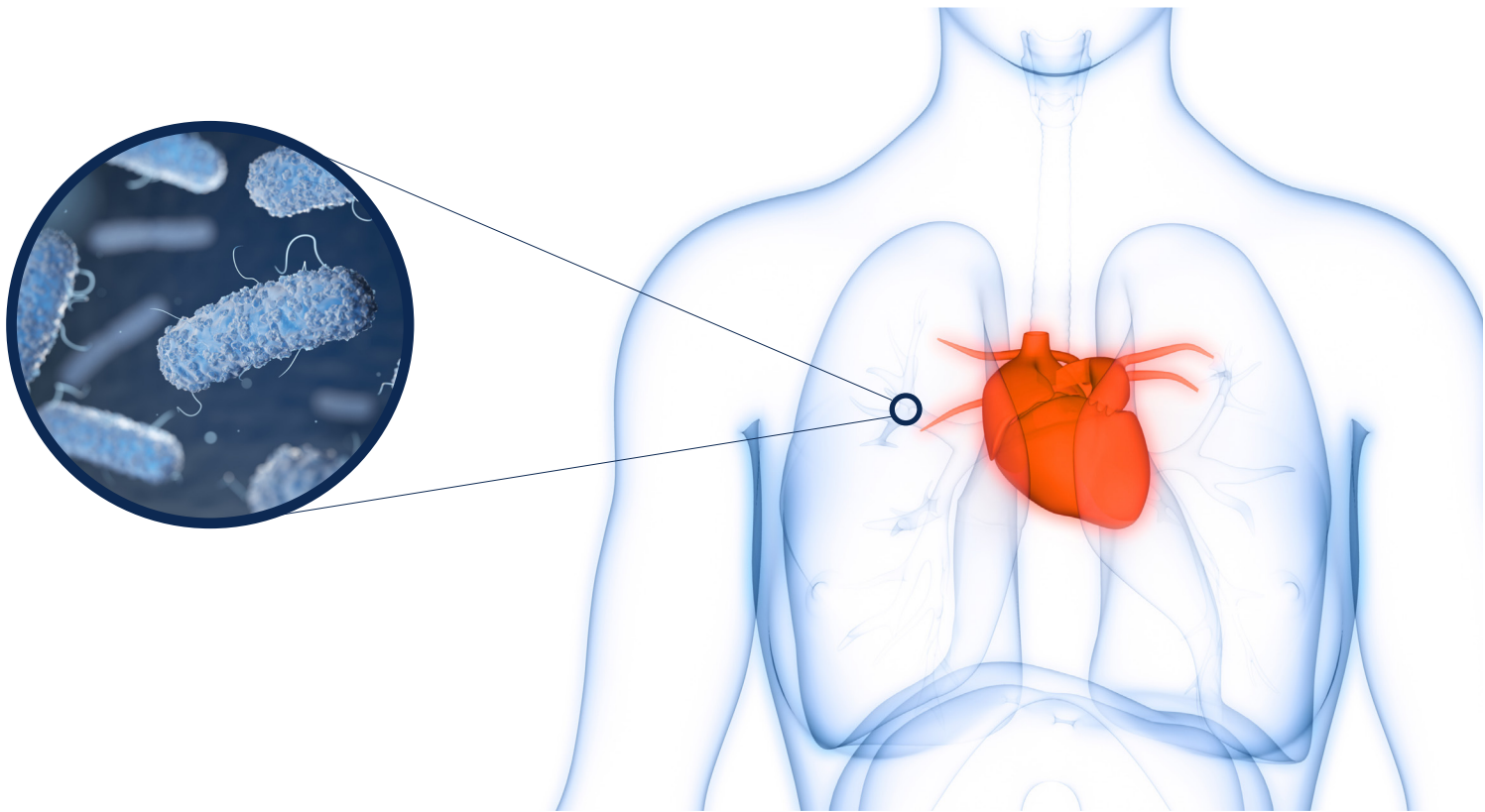
a guide to

LEGIONELLA TESTING



Health Impacts

Legionella bacteria, the causative agent of legionellosis, are commonly found in fresh water including manmade aquatic systems such as cooling towers, indoor plumbing, pools, and other water features. When the bacteria are aerosolized and inhaled they can cause infections with symptoms of an atypical pneumonia. There are two distinct forms of legionellosis - **Pontiac Fever** and the more severe form of **Legionnaires' disease (LD)**. In particular, people with compromised immune systems or respiratory disease, smokers, and the elderly are considered at high risk of contracting disease from exposure to *Legionella* bacteria. In a review of LD cases from 2000 – 2014, the Centers for Disease Control (CDC) suggested that 9 out of 10 cases could have been prevented with a more effective water management program.



Sources of *Legionella*



Cooling Towers



Indoor Plumbing



Other Water Features

Water Management

The CDC toolkit provides information on developing a water management program to reduce *Legionella* growth and spread in buildings. It references ANSI/ASHRAE Standard 188 which was published by the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE). The standard establishes minimum legionellosis risk management requirements for building water systems. It applies to commercial, institutional, residential and industrial buildings excluding single-family homes. The basic outline of a water management program can be summarized in 7 steps starting with the formation of a management team which is responsible for planning, implementation and documentation of the program.

Most water management programs will describe and define critical control points that allow for sampling, testing and application of control measures. Testing for *Legionella* bacteria is typically performed via spread-plate culture as outlined in ISO11731 and by the CDC. Other testing methods include culture testing by Legiolert[®] (IDEXX), quantitative Polymerase Chain Reaction (qPCR) and procedures based on serological assays. While each test method has advantages and disadvantages concerning sensitivity, specificity and turn-around-time, the spread-plate culture is considered the reference method and “gold standard” for enumeration.

Water Management Program

1. **Establish** management team
2. **Describe** building water system
3. **Identify** areas where *Legionella* could grow
4. **Decide** where control measures are applied and how to monitor them
5. Establish ways to **intervene** when control limits are not met
6. Make sure the program is **effective**
7. **Document** and communicate all activities

9 out of 10

cases could have been **prevented** with a **more effective water management** program.



Testing Services

Legionella - Traditional Culture (CDC Method)

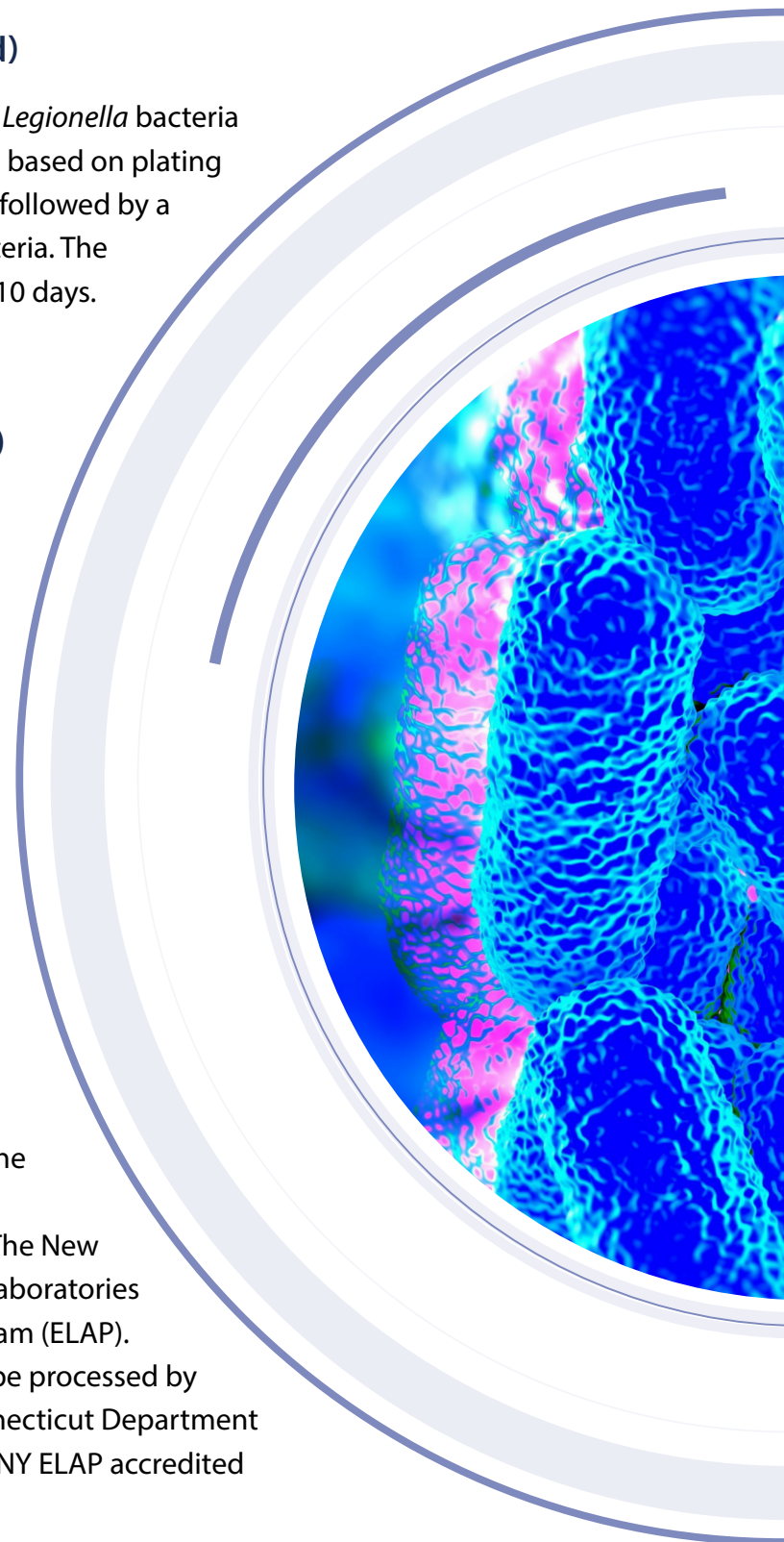
The CDC published instructions for the recovery from *Legionella* bacteria from environmental sources in 2005. The procedure is based on plating filter concentrated water samples onto selective agar followed by a series of confirmation steps to identify *Legionella* bacteria. The traditional culture method requires incubation of 7 – 10 days. Water, swab and air are acceptable sample types.

Legionella - Traditional Culture (ISO Method)

The international reference method ISO11731:2017 describes isolation and enumeration of *Legionella* bacteria via traditional spread-plate culture of concentrated water samples. Samples are heat- and acid-treated to reduce background bacteria. In addition, a portion of non-treated sample is plated for culture testing. Suspect colonies are identified as *Legionella* through a series of confirmatory steps. The traditional culture method by ISO11731 requires incubation of 10 days. Water and swabs are acceptable sample types.

Legionella - Traditional Culture (ISO Method, NY ELAP Accredited)

Our NY ELAP accredited culture method is based on the same protocol as the ISO method and uses traditional spread-plate culture of concentrated water samples. The New York State Department of Health audits and certifies laboratories via the New York State Environmental Approval Program (ELAP). All samples for compliance testing in New York must be processed by the NY ELAP accredited method. In addition, the Connecticut Department of Public Health requires certification and recognizes NY ELAP accredited laboratories for CT State certification.



Legionella - PCR Testing (iQ-Check®)

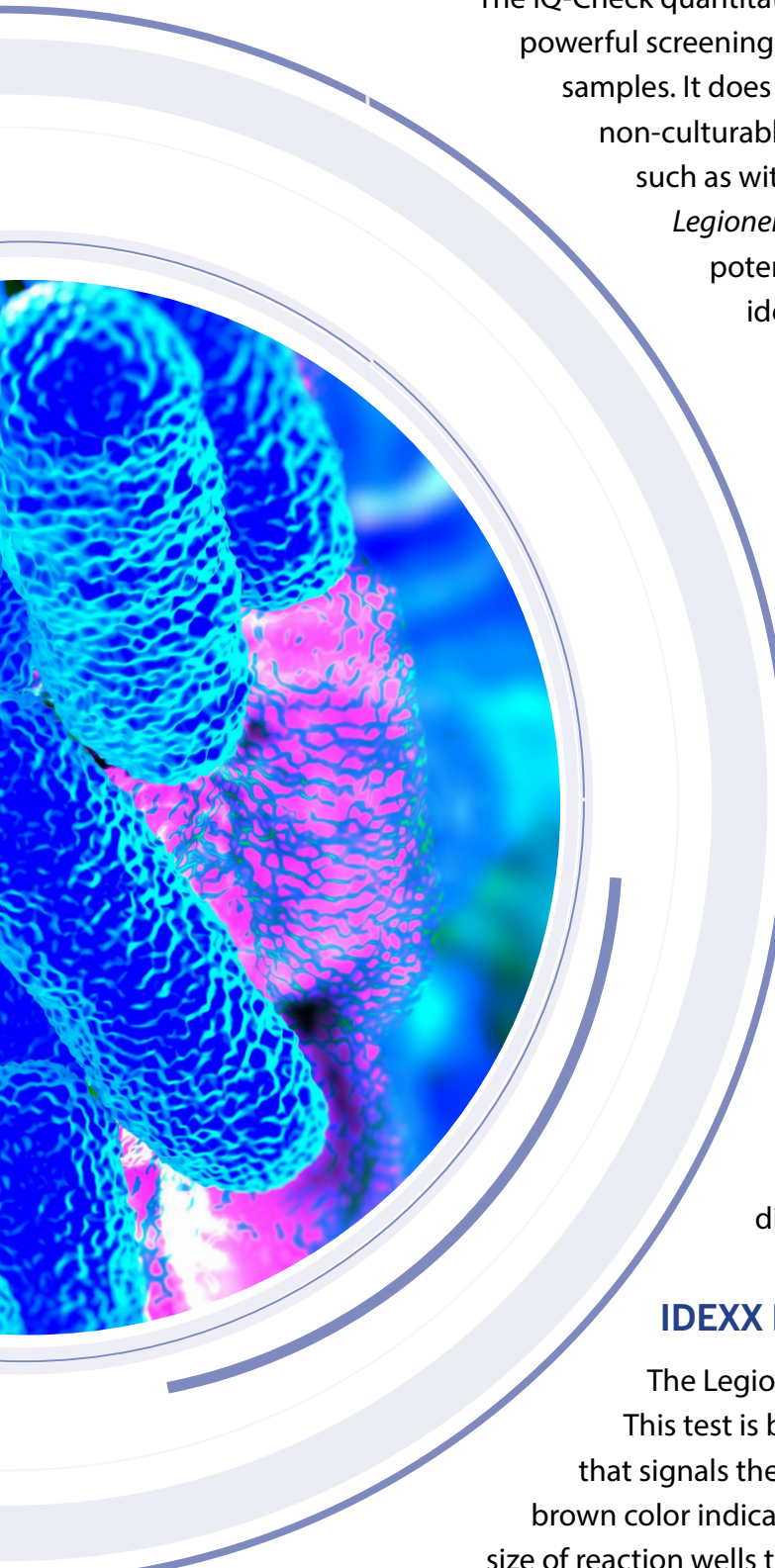
The iQ-Check quantitative polymerase chain reaction (qPCR) provides an extremely powerful screening tool for the rapid *Legionella* detection in environmental samples. It does not distinguish between living and dead and/or viable but non-culturable cells. Unless the environment has been recently altered, such as with a biocide application, moderate to high populations of *Legionella* detected by qPCR are usually indicative of an existing or potential future problem. Therefore, the PCR method can rapidly identify potential sources, facilitating disinfection processes and help to prevent further exposures. Currently, there is no reliable correlation or conversion factor between the enumeration in qPCR and the number of colony forming units in culture testing. However, negative qPCR results are an excellent predictor for negative culture samples.

Legionella – PCR Triplex (Nephros)

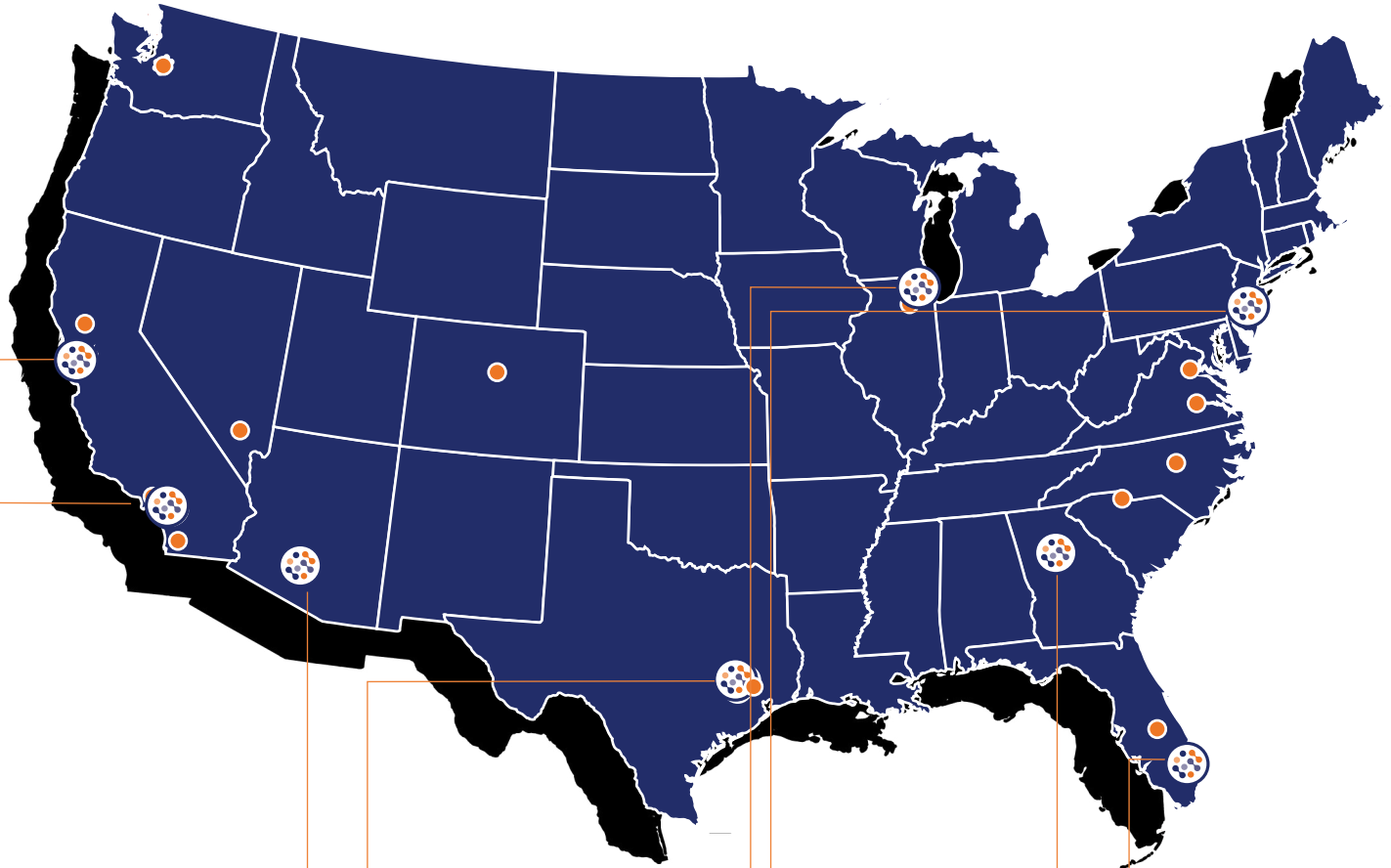
The triplex PCR test has been implemented specifically for the purpose of screening water samples for routine monitoring. The test allows differentiation between *Legionella pneumophila* serogroup 1, *Legionella pneumophila* all serogroups and all common *Legionella* species. Only one 250 ml sample is required for fast screening by qPCR and follow-up for PCR-positive samples via culture test to determine action levels when warranted. Similar to the iQ-Check system, this test is an excellent predictor for negative culture samples and does not differentiate between viable and non-viable cells.

IDEXX Legiolert®

The Legiolert Test detects *Legionella pneumophila* in water samples. This test is based on a bacterial enzyme detection technology that signals the presence of *L. pneumophila* through production of a brown color indicator. Enumeration is determined via the number and size of reaction wells that undergo color change. The Legiolert test detects *L. pneumophila* at 1 organism in 100 mL within 7 day incubation.



Eurofins Built Environment Locations are Central to High Risk Zones



Tustin, CA

2841 Dow Avenue
Suite 100
Tustin, CA
(714) 895-5494

Houston, TX

6110 W. 34th Street
Houston, TX
(713) 290-0221

Marlton, NJ

3000 Lincoln Drive E.
Suite A
Marlton, NJ 08053
(866) 871-1984

Fort Lauderdale, FL

6301 NW 5th Way #1410
Fort Lauderdale, FL
(866) 871-1984

San Francisco, CA

6000 Shoreline Court #205
South San Francisco, CA
(866) 888-6653

Phoenix, AZ

1501 W. Knudsen Drive
Phoenix, AZ
(800) 651-4802

Chicago, IL

1815 Diehl Road #800
Naperville, IL
(866) 871-1984

Atlanta, GA

6215 Regency Parkway
Norcross, GA
(877) 711-8400



Eurofins *Legionella* Laboratory

● Eurofins Built Environment Testing Lab

Frequently Asked Questions

1 WHICH METHOD SHOULD I USE FOR TESTING?

The standard method for risk-management of *Legionella* bacteria in water systems is culture testing following ISO11731:2017. A very similar culture method has been described by the Centers for Disease Control (CDC) in 2005. The intent of the CDC publication was to provide a method for the recovery of *Legionella* isolates for case investigations. Both culture methods are commonly referenced and applied in the industry to detect and enumerate *Legionella* bacteria. Other alternative methods may be used with specific goals in mind, for example qPCR to get results faster or to quickly identify negative water sources.

2 IS THERE A SAFE LEVEL FOR *LEGIONELLA*?

No – there is not a safe concentration level for *Legionella* bacteria. There are several risk factors that influence and increase the likelihood of infection including the pathogenicity of the *Legionella* strain, sensitivity of the individual and the mode of transmission or ability to enter the lungs. For example smokers, patients with COPD or compromised immune systems, and the elderly are considered high-risk groups and will be more likely to contract the disease. There are some guidelines on *Legionella* risk-management as well as local legislations (e.g. in New York) that do include action levels for certain water sources. However, there is no level that is considered safe and without any risk of infection.

3 HOW MUCH WATER DO I NEED TO SAMPLE?

For routine monitoring with the purpose of risk-management, 250ml is a sufficient sample volume. In an outbreak situation and in the context of case investigations, the CDC recommends sampling 1 liter or more. The sensitivity of the test is influenced by a variety of factors of the testing procedure. Different laboratories may produce different limits of detection depending on processing parameters. For water sources that are expected to contain high concentrations of bacteria (e.g. cooling towers) sample volumes of less than 250ml may be appropriate.

4 DO I NEED TO SHIP SAMPLES ON ICE?

Legionella bacteria are predominantly viable but dormant at temperatures under 20°C (68°F). According to ISO guidelines, the recommendation on temperatures for transport and storage for bacterial testing is to keep water samples between 2°C and 8°C. With those parameters in mind and in consideration of seasonal outside temperatures, we recommend the use of cold packs or ice with *Legionella* samples. Also, send samples as soon as possible after sampling is completed and use overnight shipping.

5 HOW DO I APPLY ACTION LIMITS FOR qPCR?

Quantitative polymerase chain reaction (qPCR) offers a fast and sensitive detection of *Legionella* bacteria. The results are reported in genome units (GU) per liter. There is not a direct conversion of GU to colony forming units (CFU). Most qPCR systems will detect DNA from viable and from dead cells. In many cases, live cells may not produce a colony under the process conditions of the culture test. Those cells are considered to be viable but non-culturable (VBNC). We expect the qPCR results to produce a significantly higher number of GU compared to the number of CFU in a given samples. The consensus is that the qPCR is a very sensitive method that predicts negative results with high confidence, meaning that qPCR negative samples are usually also negative for culture testing. qPCR positive samples can be followed up by a culture test for threshold application.



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