Routine Testing for *Legionella*

Purpose

Use this document to:

1. Help analyze hazards and establish *Legionella* control measures per ASHRAE Guideline 12
2. Complement existing resources for testing, sampling, and water management programs (WMPs)
3. Support environmental assessment during public health investigations

Testing for public health investigations must always be performed in conjunction with the authority having jurisdiction (AHJ). The below guidance is for routine testing only.

Testing Objectives

Testing may be useful for routine and non-routine purposes, such as:

- Establishing a baseline measurement for performance indicators
- Validating a WMP
- Evaluating potential growth and transmission sources
- Confirming success or failure of remedial treatment
- Investigating potential sources of environmental exposure for persons with disease

Routine testing may be particularly beneficial for certain types of facilities, such as:

- Facilities that house or treat individuals at increased risk for Legionnaires' disease (e.g., senior communities, outpatient clinics)
- Facilities unable to meet control limits consistently
- Facilities with a history of associated Legionnaires' disease cases

Sample Collection

1. Perform an environmental assessment to identify areas with increased risk of *Legionella* growth and spread. Consider the key factors for *Legionella* growth (i.e., sediment and biofilm, temperature, water age, and disinfectant residual) when assessing risk.

Before sampling, consider how results will be used in the broader context of a water management program.

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2. Create a sampling plan that represents the entire building water system. Sampling location recommendations are included in the CDC Sampling Procedure and Potential Sampling Sites.
3. The volume of water you collect may depend on the source type (potable vs. non-potable) or condition (detectable disinfectant residual vs. visible debris and no detectable disinfectant residual). Typically, a 250 mL sample is sufficient for routine testing. Larger sample volumes and other sample types, such as swabs or ice, may provide additional information for at-risk facilities.
4. Reference CDC Sampling Procedure and Potential Sampling Sites for step-by-step instructions on selecting sites and collecting samples.

Test Methods and Laboratory Considerations

Some test methods may be performed onsite by the user or a qualified technician, while other methods may require contracting with a commercial laboratory. Regardless of the test method, be sure that you understand the performance characteristics of the test such as sensitivity, specificity, and limitations. For best results, follow instructions from the manufacturer or testing laboratory closely.

Consider testing for all *Legionella* species as all are supported by similar environmental conditions.

Considerations when working with laboratories testing for *Legionella*:

- Accreditation by a regional, national, or international accrediting body to a recognized standard for routine *Legionella* test methods, such as ISO/IEC 17025
- Capability of retaining *Legionella* isolates from samples for additional characterization
- Capacity to perform additional *Legionella* characterization as needed by the submitter

Test Methods

Traditional Culture (spread plate)	Nucleic Acid Amplification Test	Alternative and Novel Methods
• Detects viable bacteria • Detects all *Legionella* species • Results typically reported in colony forming units (CFU) per volume with limit of detection ~10 CFU/mL • Yields isolate for additional characterization • Results typically reported in 7–14 days • Is subject to skill, experience, and procedural rigor of the laboratory • May be preferred for evaluating growth trends	• Detects *Legionella*-specific DNA or RNA • May not differentiate between live and dead bacteria • Results typically reported in genomic units (GU) which is not directly equivalent to CFU • Results typically reported in 2–48 hours • Is useful for negative screening • May be preferred for evaluating whether remediation was successful	• Should be validated against a standard method by a third party (e.g., ISO/IEC 13843) • Should be verified by the laboratory (e.g., per ISO 17025) • May detect only a subset of *Legionella* species or serogroups • Results may be reported in hours or days • Results may be expressed in a variety of units or only qualitatively • May be useful for repeated measurements when quick turnaround time is preferred

Note: Test method may vary by the type of water system and the reason for testing.

Sample volumes processed, plate types, resuspension procedures, and result reporting vary by lab even when using standard operating procedures such as CDC methods or ISO 11731 from the International Organization of Standardization.

Test Results for WMP Performance

Results of *Legionella* testing alone do not provide a measure of health risk and are not predictive of disease. **There is no known safe amount or type of *Legionella*.** Additional considerations for results are:

- Results indicate the presence of *Legionella* within the sample only, as there is variability across water systems.
- Sample handling, transport, and lab processing can affect results.
- Results have been interpreted based on concentration (e.g., CFU/mL), extent of colonization (e.g., % positive), and type of *Legionella* (e.g., *Legionella pneumophila* serogroup 1 vs other species, serogroups, or sequence types). **See Figure 1 for a multifactorial approach to interpreting *Legionella* test results as performance indicators.**
- The presence of any *Legionella* should trigger response activities (see "Suggested Response Activities").

Performance Indicators and Suggested Response for Routine *Legionella* Test Results

If test results are expressed in units other than CFU/mL, consult the testing laboratory or test manufacturer for appropriate result interpretation.

- If <1 CFU/mL for potable water or if <10 CFU/mL for cooling towers, *Legionella* growth appears well controlled
  - Continue Program
- If ≥1 CFU/mL for potable water or if ≥10 CFU/mL for cooling towers, conditions may allow for *Legionella* growth
  - Implement Suggested Response Activities
- If 10 to 100-fold increase for potable water or cooling towers, *Legionella* growth appears to be poorly controlled
  - Implement Suggested Response Activities
- If >100-fold increase, *Legionella* growth appears to be uncontrolled
  - Implement Suggested Response Activities
- Due to their ability to rapidly grow and spread *Legionella*, any detection of viable *Legionella* in a hot tub or decorative fountain should prompt a response and corrective actions

Suggested Response Activities

Suggested activities to be implemented when *Legionella* laboratory results are not indicative of well-controlled growth per performance indicators above:

1. Review sample collection, handling, and testing for potential errors.
2. Confirm that system equipment is in good working order and functioning as intended.
3. Review records to confirm that the WMP was implemented as designed (verification).
4. Review assumptions about operating conditions, such as physical and chemical characteristics of incoming water.
5. Re-evaluate fundamental aspects of the WMP, including analysis of hazardous conditions, cleaning, maintenance procedures, chemical treatment, and other aspects that could affect *Legionella* testing.
6. Adjust WMP as necessary to address any deficiencies identified.
7. Consider whether remedial treatment is needed only after completion of the above.
8. If remedial treatment was performed, wait at least 48 hours after the system returns to normal operating conditions and retest a set of representative samples to confirm the effectiveness of the response.

If *Legionella* growth does not appear well controlled in healthcare facilities or facilities with populations at increased risk for Legionnaires' disease, consider implementing immediate control measures to protect people from exposure to water aerosols while implementing the guidance above. Note, if the root causes of *Legionella* growth are not identified and controlled, *Legionella* growth is likely to reoccur.

Whenever a case of disease is associated with a water system as determined by the public health AHJ, always:

- Review WMP verification and validation activities
  - Verification: Are the WMP activities occurring as intended?
  - Validation: Are the WMP activities working as intended and effective for *Legionella* control?
- Re-evaluate and revise WMP if needed

Resources

- Toolkit for Controlling *Legionella* in Common Sources of Exposure: https://www.cdc.gov/control-legionella/php/toolkit/control-toolkit.html
- Toolkit: Developing a Water Management Program to Reduce *Legionella* Growth and Spread in Buildings: https://www.cdc.gov/control-legionella/php/toolkit/wmp-toolkit.html
- *Legionella* Environmental Assessment Form: https://www.cdc.gov/investigate-legionella/Legionella-Environmental-Assessment-Form.pdf
- PreventLD Training: https://www.cdc.gov/control-legionella/php/training/index.html
- ASHRAE Guideline 12: https://www.ashrae.org/technical-resources/standards-and-guidelines/guidance-for-water-system-risk-management
- Sampling Procedure and Potential Sampling Sites: https://www.cdc.gov/investigate-legionella/media/pdfs/cdc-sampling-procedure.pdf
- Implementing Control Measures in Healthcare Facilities: https://www.cdc.gov/investigate-legionella/php/healthcare-resources/control-measures.html

Figure 1. Routine *Legionella* testing: A multifactorial approach to performance indicator interpretation*°Δ

Concentration indicates that *Legionella* growth appears:

Uncontrolled	Poorly Controlled	Well Controlled			
≥10 CFU/mL† in potable water **OR** ≥100 CFU/mL in cooling towers	1.0–9.9 CFU/mL in potable water **OR** 10–99 CFU/mL in cooling towers	Detectable to 0.9 CFU/mL in potable water **OR** Detectable to 9 CFU/mL in cooling towers	No *Legionella* detected in a single round of testing	No *Legionella* detected in multiple rounds of testing	No *Legionella* detected in multiple rounds of testing with methods that detect viable and non-viable bacteria of any *Legionella* species

Change in concentration over time indicates that *Legionella* growth appears:

Uncontrolled	Poorly Controlled	Well Controlled			
100-fold or greater increase in concentration (e.g., 0.05 to 5 CFU/mL)	10-fold increase in concentration (e.g., 0.05 to 0.5 CFU/mL)	*Legionella* concentration steady (e.g., 0.5 CFU/mL for two consecutive sampling rounds)	No *Legionella* detected in a single round of testing	No *Legionella* detected in multiple rounds of testing	No *Legionella* detected in multiple rounds of testing with methods that detect viable and non-viable bacteria of any *Legionella* species

Extent indicates that *Legionella* growth appears:

Uncontrolled	Poorly Controlled	Well Controlled			
Detection in multiple locations **AND** a common source location‡ **OR** Detection across many locations within a water system	Detection in a common source location that serves multiple areas **OR** Detection in more than one location within a water system	Detection in a few of many tested locations within a water system	No *Legionella* detected in a single round of testing	No *Legionella* detected in multiple rounds of testing	No *Legionella* detected in multiple rounds of testing with methods that detect viable and non-viable bacteria of any *Legionella* species

Type¥ of *Legionella* (species and serogroup) associated with Legionnaires' disease:

Highly Associated	Less Associated
L. pneumophila serogroup 1; Non-Lp1 *L. pneumophila*; Presence of multiple different *Legionella* species or serogroups	Any non-*pneumophila Legionella* species including "blue-white" fluorescent *Legionella*

* This figure is intended for use during routine testing of potable water and cooling towers only. Due to their ability to rapidly grow and spread *Legionella*, any detection of viable *Legionella* in a hot tub or decorative fountain should prompt a response, including a review of the water management program and corrective actions. Test results are performance indicators and are not a measure of risk of human illness. This figure is not intended for use if a building or device is associated with Legionnaires' disease (LD) cases or an outbreak.

° See "Routine testing for *Legionella*" for guidance regarding suggested response activities. Comparable results may lead to different suggested response activities when other factors are considered (e.g., if there is evidence of poorly controlled growth at a healthcare facility).

Δ Considering the type of *Legionella* identified along with other *Legionella* testing performance indicators provides a clearer picture of water system control than the results of any single indicator. For example, facility owners and operators may consider implementing immediate interventions for a healthcare facility with: A. detectable but <10 colony-forming units per milliliter (CFU/mL), B. non-Lp1 *Legionella pneumophila*, C. observed at steady concentrations, but D. detected at multiple distal locations including a central water heater.

† Concentrations expressed as CFU/mL are for test results generated by traditional spread plate culture methods. If other test methods are used, consult testing lab or manufacturer instructions for appropriate interpretation.

‡ Common source location examples include water heaters, hot water returns, storage tanks, and cooling tower basins.

¥ If a facility has a history of associated LD cases, then sequencing isolates obtained during routine testing may provide performance indicators regarding outbreak strain persistence (if that strain is detected).

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