Routine Testing for *Legionella*

**Purpose**

Use this document to:

1. Help analyze hazards and establish *Legionella* control measures per ASHRAE Guideline 12-2020
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.*
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 for Investigations.

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.


### 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.

**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

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

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

**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

- PreventLD Training: https://www.cdc.gov/nceh/ehs/elearn/prevent-LD-training.html
- Immediate Control Measures for Healthcare Facilities: https://www.cdc.gov/legionella/health-depts/healthcare-resources/cases-outbreaks.html#measures-facilities