EMSL ANALYTICAL, INC.
LABORATORY • PRODUCTS • TRAINING

www.legionellatesting.com

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NYSDOH ELAP Legionella
Certified Labs:
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- Cinnaminson, NJ

United States and Canada Locations

- EMSL Laboratory
- EMSL Service Center
- LA Testing Laboratory

Headquarters
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What Is Legionella?

The *Legionella* organism is a Gram negative, rod shaped bacterium that can cause pneumonia (Legionnaires’ disease) or a flu-like illness (Pontiac fever). It was first identified and recognized as causing disease during the outbreak that occurred in conjunction with the American Legion Convention in Philadelphia in 1976. There are over 50 species of *Legionella* and 18 of those can cause disease. It is thought that the species *Legionella pneumophila* causes most of the infections.

*Legionella* is a fastidious organism, meaning it has specific growth requirements that need to be met in order for it to survive and grow. Some of these growth requirements are:

- Temperature above 68° F
- Iron
- L-Cysteine
- Biofilm (particularly protozoans)

Unlike some other bacteria, it can survive at lower temperatures (even below freezing), at lower dissolved oxygen levels and is somewhat resistant to chlorine disinfection. Certain plastics and organics can provide nutrients for growth. These attributes make our modern day plumbing systems a good habitat for the organism.

**Legionella Overview**

Common infections caused by *Legionella* are Legionnaires’ disease (LD), a severe pneumonia, or Pontiac Fever, a flu-like illness. However other species (i.e., *L. micdadei*, *L. longbeachae*) can cause LD as well. *L. pneumophila* has many subgroups called serotypes. *L. pneumophila* serotypes 1, 3, 5, and 6 have been the causative agents of Legionnaires’ Disease. Co-infections with different species and/or serotypes have occurred.

**Ecology**

*Legionella* are commonly found in aquatic environments and some species have been found in soil. The organisms are found in a wide range of environmental conditions and are relatively resistant to low pH, dissolved oxygen levels, and
routine chlorination techniques for drinking water. Temperatures above 104°F promote rapid multiplication of the organism. The organisms are consistently found in the biofilm that forms in aquatic environments, cooling towers and potable water systems.

**Epidemiology**

The health risk factors for legionellosis are people who are immunocompromised by an underlying medical condition, those taking immunosuppressive drugs, heavy smokers, those who have chronic lung conditions, and the elderly. Several studies have documented cases of pediatric legionellosis in children under 1 year of age or children with underlying medical conditions such as malignancy or immunosuppression. Legionellosis is not contagious; there is no evidence that the disease can be transmitted from person to person. Exposure must be through inhalation or aspiration of contaminated, aerosolized water. Once a person has Legionnaires’ disease, getting it a second time is extremely rare.

**Monitoring Guidelines**

As a result of the Legionnaires’ disease outbreak that occurred in the Bronx in August of 2015, both the State of New York Department Health and New York City Department of Health and Mental Hygiene have passed the first US regulations specifically for testing cooling towers (and drinking water water systems in hospitals) for *Legionella*. These regulations specify action levels requiring cooling tower disinfection. In addition the US Centers for Disease Control and Prevention (CDC) recommends routine monitoring for *Legionella* in all bone marrow and organ transplant hospitals nationwide. Routine monitoring in healthcare facilities is recommended or required in several states such as NY, TX, MD, Los Angeles County and Allegheny County PA. Canada has guidelines for monitoring healthcare facilities. In June, 2015 The American Society of Heating, Refrigeration and Air Conditioning Engineers (ASHRAE) passed ASHRAE Standard 188 for the prevention of Legionellosis in buildings. While this voluntary standard of practice doesn’t specifically address *Legionella* testing, it offers basic recommendations for establishing a building water system safety plan.
Sampling And Analysis
Take a 1000mL sample for potable water and a 250mL sample for non-potable water. Be sure to use sterile bottles with a chlorine neutralizing agent. Since biofilms are the actual reservoirs for the bacteria it is also recommended to take sterile swab samples of biofilm in areas where it is present. Samples should be shipped overnight to the lab on freezer packs. Culturable analysis either by the US Center for Disease Control or the International Standard Organization is the “gold standard” and requires 10-14 days. Testing by Polymerase Chain Reaction (PCR) takes 2-3 days and may be very useful for providing fast, presumptive results to reduce liability during an outbreak. Testing by Next Generation DNA Sequencing can provide evidence that compares the molecular fingerprint of an environmental isolate to a clinical isolate. Isolating *Legionella* from environmental samples is difficult. Make sure to use an experienced lab that is either CDC ELITE or HPA (United Kingdom) proficient.

An Overview of *Legionella* Analyses

Background
The first recognized outbreak of Legionnaires’ Disease occurred in the US at the American Legion Convention in Philadelphia during the summer of 1976. There were several hundred people who were stricken. Thirty four people died from the disease. As a result of the efforts of the US Centers for Disease Control and Prevention (CDC), this was the first time the bacteria was cultured and identified. Earlier outbreaks of the disease went undiagnosed. Since that time, there have been many identified outbreaks in this country and abroad prompting professional organizations and health departments worldwide to implement guidelines for diagnosing and reporting the disease, and monitoring the organism. However, there are no health standards for safe levels of exposure to Legionella.
Transmission and Epidemiology

Ubiquitous in all aquatic environments, *Legionella* bacteria are found in groundwater as well as fresh and marine surface waters. The bacteria enter our plumbing systems, whirlpool spas, and cooling towers via these water sources. Unless control measures are conducted properly and routinely, the biofilm, scale, and corrosion that builds up over time in these systems will protect the organism and allow it to multiply.

Contaminated aerosolized water from cooling towers, whirlpool baths, nebulizers, faucets, and showerheads becomes airborne. When a susceptible host inhales the contaminated aerosol, legionellosis can occur. Aspiration of the contaminated water can also cause the disease. Legionellosis can cause two types of illness: 1. a severe form of pneumonia (Legionnaires’ Disease) often accompanied by serious long term health effects, and 2. a mild flu-like illness called Pontiac Fever. Other infected organs, and asymptomatic infections may also occur.

Historically, risk factors for getting the disease included age, gender (males), compromised immune systems, and pre-existing medical conditions such as chronic obstructive pulmonary disease, cancer, diabetes, and the use of rheumatoid arthritis drugs and chemotherapy. Men over 65 years of age who were heavy smokers and drinkers were identified as being at greatest risk. While that is still true, recent research from Neil and Berkelman at Emory University has identified an abrupt increase in the incidence of Legionnaires’ Disease in the US in all age groups in the last 20 years. This trend has also been noted internationally by other researchers. They have noted an overall increase in the disease among all people aged 45 to 64. Rates of disease in males still exceed the rates in females.

There have also been cases of the disease in healthy, younger people. Premature, immuno-compromised, or ventilated neonates are at risk from hospital-acquired infection. In addition, cases have been reported in children aged 15-19 years old. Although the disease is under-reported, travel (cruise ships), hotel, and resort related outbreaks are reported each year. These are mostly associated with the use of whirlpool spas and stagnant potable water. While community-acquired
outbreaks involving cooling towers and whirlpool spas receive the most media attention, studies prove that building potable water sources account for most of the infections. This is particularly true in hospitals and nursing homes where there are large numbers of immunosuppressed or critically-ill people. For these reasons, many state health departments have guidelines that recommend routine monitoring for *Legionella* in critical-care hospitals and nursing homes. In 2008, the Veteran’s Administration promulgated a directive which requires all VA hospitals and rehabilitation centers to implement monitoring for the bacteria in their potable water systems.

**Choosing Sampling Methods**

Proper methods for collecting and analyzing samples are necessary to ensure defensible results. Since the bacteria in water are present in very low levels, 1000 mL potable water samples are recommended by the US Centers for Disease Control and Prevention (CDC). This sample size allows for the bacteria in the water to be concentrated, allowing for better detection in potable water samples. Many professional guidelines recommend semi-annual sampling for potable water sources.

In non-potable water sources such as cooling tower water, a 250 mL sample size is sufficient. Professional guidelines suggest these sources be monitored quarterly.

Sampling should be conducted in a way that maximizes recovery of the organism. *Legionella* samples should be collected wherever water aerosolization may occur.

Sampling aerosolized water alone, however, will likely miss the real source of the organism. This source is the biofilm or slime that is often found in our plumbing systems, cooling towers, and whirlpool baths.

There is no correlation between Heterotrophic (aerobic) plate counts (HPC/APC) and *Legionella*. Therefore, HPC testing should not be used as a surrogate for *Legionella* testing. Routine *Legionella* testing will identify a potential exposure risk. The goal is to verify a history of non-detectable *Legionella* results over time.
Where to Look for *Legionella*

When conducting your building water system investigation, walk-through, or risk assessment use this section to help you identify all potential *Legionella* reservoirs in the building. If conducting an investigation due to a suspected case or outbreak, also survey the surrounding neighborhood to identify any cooling towers, wastewater treatment facilities, storm water/gray water re-use facilities (i.e. golf course spray irrigation water systems), or ornamental fountains that may be located near the building in question. If necessary, obtain permission to sample these off-the-property locations. Also identify and visually inspect all the building fresh-air intakes/pedestrian walkways with respect to these neighborhood locations.

1. Potable water systems
2. Cooling towers
3. Water walls
4. Aerosol generation during the biological treatment of some industrial process wastewater streams ie., pulp and paper manufacturing, food and beverage manufacturing, pharmaceutical manufacturing
5. Aerosol generation during municipal water and wastewater treatment
6. Raw, utility or fire water
7. Ornamental outdoor and indoor water fountains and ponds
8. Heated swimming pools
9. Hot tubs
11. Metal working fluids
12. Medical therapy equipment like dialysis units, nasogastric tubes, respiratory equipment and nebulizers, whirlpool baths
13. Commercial car wash facilities particularly those using recycled water
14. Supermarket vegetable misters
15. Ice machines in hotels and hospitals
16. Outdoor body misters at ballparks and amusement parks
17. Use of tap water in place of manufactured windshield cleaner fluid
18. Fog Machines
19. Ultrasonic Dental Descalers
20. Storm water/gray water spray irrigation systems
**Legionella Sampling Locations**

Sampling is the single most important activity for every project. The purpose of sampling is to identify the goals of the project, to answer specific questions, or to develop a hypothesis to test. While it is normal for clients to be upset when they encounter a suspected case or an outbreak, avoid taking random samples without understanding how the data will be used and who could ultimately see the results.

Here are some key questions to consider that will help you focus your sampling efforts:

1. **What are the appropriate test methods and the level of detail needed for your project?** (See test codes)
2. **Where do you need to sample?** Are there particular types of equipment or devices that need to be sampled?
3. **When do you need to sample?** Consider there may be times to take cooling tower samples in the summer and early fall to account for worst case scenarios rather than taking strictly periodic samples.
4. **How many samples do you need to take?**

It is important to take as many samples as necessary to obtain sufficient data to answer your questions. Every sampling location should provide data to answer a question, however avoid taking more samples than necessary. Understand that one sampling point or one sampling event will not constitute a representative number of samples and will not pass a legal challenge. The purpose of sampling will vary according to different project goals so adjust your sampling strategies accordingly. Use the “Where to Look” and “Sample Locations” sections to help focus your activities.

If you represent a Veterans Administration Medical Center, understand the required test codes and sampling locations before doing any sampling. Review the VHA Directive dated August 2014 which can be found at [http://www.va.gov/vhapublications/ViewPublication.asp?pub_ID=3033](http://www.va.gov/vhapublications/ViewPublication.asp?pub_ID=3033)
Cooling Tower Sampling Locations

*Sample Quarterly*
- Tower Makeup
- Tower Sump away from makeup* (water and swab)
- Inlet to Heat Exchanger
- Distribution Pack
- Outlet from Heat Exchanger
- Tower Pack* (water and swab)

Potable Water Sampling Locations

*Sample Semi-Annually*
- City Water Main Entry Point
- Storage Tanks
- Hot Water Heater Drain Point*
- Hot Water Return
- Last Point on Cold Water/First Point on Hot Water
- Last Point on Hot Water*/First Point on Cold Water
- 10% of Selected Outlets (water and swab)

*Test Routinely. Test all locations to establish a baseline.
Test all locations during an outbreak.*
Sampling Instructions - *Legionella*

1. Personal safety and precautions should be observed during sampling. Avoid breathing aerosols that may be contaminated with *Legionella* bacteria. Avoid generating aerosols or water mists during sampling of the water system. Wear a respirator equipped with an N95 respirator, a HEPA cartridge, goggles, and sterile nitrile gloves.

2. Prepare or obtain sterile, screw-capped plastic bottles for sampling. Sodium thiosulfate is routinely added to the bottle as a preservative and halogen (chlorine or bromine)-neutralizing agent.

**Recommended sample size:**

<table>
<thead>
<tr>
<th>Potable Water Sampling</th>
<th>Non-Potable Water Sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Liter Water Sample</td>
<td>250 mL Water Sample</td>
</tr>
</tbody>
</table>

3. For drinking or potable water, such as water fountains, faucets, and shower heads, collect two samples if possible. Collect the “pre-flush or first draw” sample by draining the first 1000 mL of water from the faucets or flush drains into a bottle. Allow the water to run for approximately 60 seconds and collect the second draw of 1000 mL of water. Leave a one-inch space on top of the water sample. The second sample is also called “post-flush or second draw” sample. Take cold water samples from drinking water fountains and areas that have dead legs. Also take ice samples, allowing them to melt to reach a 1000 ml sample.

4. When sampling faucet aerators and showerheads, remove the aerators aseptically. Take swabs of the inside the faucet and shower heads as far as you can reach with the swab. Swirl the swab on the inside of the pipe three times. Your swabbing procedure should be consistent between sampling locations. When sampling cooling towers, whirlpool spas or fountains, look for areas of biofilm and take a swab sample of the biofilm. We will provide sterile swabs for this purpose.
5. For non-drinking or non-potable samples from such sources as cooling towers, chillers, condensate pans, surface water in reservoirs, sprinklers, hot tubs, water walls etc., collect 250 mL water from the bottom or side of the vessel or reservoir. Leave a one inch space on top of the sample. Record any biocide used in water treatment when collecting non-drinking water. If sampling whirlpool spas, consider taking a swab sample of any biofilm as well as a sample of the sand filter.

6. Label sample number on the bottle and record on the sample data sheet. Use a distinctive number for each sample. Complete all sample information on a sample data sheet for your own record. Send a copy with the samples to the laboratory.

7. Tightly cap the bottles. Make sure that water does not leak during shipping and transporting. Taping of bottle around the cap and neck with electric vinyl tape is recommended. Place taped bottles in a clean plastic bag.

Place the samples in insulated boxes with freezer packs to protect specimens from extreme temperature fluctuations in the summer months. **NEVER USE ICE OR DRY ICE.** Stuff the box with foam chips to cushion, and seal the box securely for shipping. Send samples by overnight express carrier. Schedule sampling between Monday and Friday so that samples can be delivered to the laboratory no later than Saturday. (Consider holidays)

Contact EMSL for the shipping address to your nearest CDC ELITE LABORATORY
Phone: 800-220-3675 or Email: info@emsl.com
**Legionella Swab Sampling Instructions**

1. Ask Facilities Management to remove aerators from shower heads and faucets where you will be sampling.

2. Turn on the water for a couple of seconds to moisten the pipe, and then turn it off. Insert the EMSL swab deep into the faucet/pipe. Try to get beyond the bend and swab around the inside surface firmly without breaking the swab stem. (If there is visible biofilm on the inside of the showerhead or faucet aerator when these are removed, they can also be swabbed.)

3. Place the swab into the EMSL sterile plastic tube and add 3–5 mL of water from the same faucet to keep the swab tip moist during transport. Snap the wooden or plastic swab stem approximately 1 in. from the top of the tube. Add a drop of 0.1N sodium thiosulfate solution provided to neutralize residual disinfectants. Tighten the tube top to prevent leakage.

4. Label the tube with a unique identifier. Record the type and location of the sample on a Sample Data Sheet, and place the tube into a cooler. Take bulk water samples:

5. After the biofilm swab is collected, turn on the water and let it run for a few minutes until the water is warm but not hot. The goal is to obtain water currently in the distribution system along with any material shed from biofilm. Avoid heating water excessively (approximately 122°F or higher) since free-floating Legionella will die quickly at elevated temperatures. Collect 1 L of water from the faucet into a sterile 1 L bottle, leaving a 1 in. space at the top. Tighten the top to prevent leakage.

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**Inquire About Our NEW Legionella Swab Sampling Kit**

CDC Recommended Sampling Protocols

**Call 1-800-220-3675 or 1-866-798-1089 to order!**
Emergency Remediation - Potable Water System

Remediating *Legionella* after a case or an outbreak from potable water is no easy matter and may require several attempts before the treatment is successful for achieving non-detectable results. In order to reduce the exposure risk immediately, consider installing filters on shower heads and faucets. Also use bottled water in place of tap water for drinking and eliminate offering ice chips for hydration until the situation is brought under control.

Unfortunately due to the ecology of *Legionella* and the nature of the treatment systems, there are no permanent solutions. The best outcome will be to reduce and control the biofilm buildup where the bacteria reside and multiply by installing a permanent in-building secondary treatment system such as copper-silver ionization, monochloramine, or chlorine dioxide. These systems must be maintained properly in order to be effective.

The proper design, maintenance, and temperature of a potable water system is the first defense for preventing the amplification of *Legionella*. Maintaining hot water above 135 degrees Fahrenheit (57 °C) and cold water less than 68 degrees Fahrenheit (20 °C) and eliminating dead legs or low flow areas goes a long way for prevention. However this is not always feasible. There are several procedures that can be taken for emergency remediation, or routine treatment of a potable water system. However, it needs to be understood that these are temporary solutions since the bacteria will rebound within a few weeks. These cleaning protocols are listed below:

Heat treatment of hot water tanks and the complete water system includes raising the temperature of the system to 157 degrees Fahrenheit (69 °C). This temperature needs to be maintained in the tank for 3 hours. The hot water needs to be drawn through all the outlets at 157 degrees Fahrenheit (69 °C) starting with the outlets closest to the hot water tank. Sequentially work away from the hot water tank drawing water at 157 degrees Fahrenheit (69 °C) to all the outlets at a trickle flow rate for 3 hours. After this is completed, flush the hot water from the system and return it to normal operating conditions. If the capacity of the system is too low for heat treatment, then chlorination should be used.
Chlorination is accomplished by draining the hot water system and manually cleaning it of debris and biofilm. Remove all deposits by scraping the surfaces followed by wet vacuuming of the tank. Fill the tank with clean water and add chlorine until 50 ppm of free chlorine is obtained through all the outlets including dead legs, low use points, and risers. Soak at this level of free chlorine for 16 hours while testing to ensure that a minimum of 30 ppm free chlorine is obtained at all outlets points for the 16 hours. After this has been accomplished, flush the chlorinated water from the system and test to ensure that less than 2 ppm chlorine remains in the system.

According to the International Plumbing Code, this chlorination needs to be conducted for all new systems. Chlorination should be conducted after: major alterations, a system tests positive for *Legionella*, a major outage, a water main break, the municipal water system has been flushed.

Continuous, proper, routine maintenance and treatment is the only way to prevent the amplification of *Legionella*.

**Emergency Remediation - Cooling Towers**

Remediating cooling towers is successful as long as the treatment is conducted in conjunction with an ongoing maintenance program of the tower. Currently, the cleaning protocols for cooling towers can be found in the American Society of Refrigeration, Air Conditioning and Heating Engineers (ASHRAE), the Wisconsin Emergency Protocol (later withdrawn by Wisconsin but the revised version can still be found in OSHA *Legionella* Technical Document), and UK Health and Safety Executive Directive for Water Treatment HS (G) 70. These protocols emphasize the need for routine maintenance, inspection, manual cleaning of system components and water treatment by professionals. Minimizing biofilm, scale, corrosion, algal growth, and sediment accumulation in the cooling tower components are critical for preventing amplification of *Legionella*. 
One approach for cleaning is a modification of the UK Water Treatment Method HS (G) 70 and includes the following:

- Chlorinate to 5-10 ppm for 5 hours with biodispersant; test for chlorine residual every 30 minutes
- Completely drain the system
- Manually clean the sump, tower pack, distribution system and drift eliminators to remove all deposits. Multi-celled systems can be cleaned sequentially.
- Refill the system.
- Chlorinate to 5-10 ppm for 5 hours with biodispersant; test for chlorine residual every 30 minutes
- Completely drain the system again; refill the tower
- Re-sample for *Legionella* after 2 weeks.

For systems having existing online chlorination, this first response is used to reduce a positive *Legionella* result:

- Maintain 5-10 ppm chlorine for 24-48 hours using a biodispersant
- Re-sample for *Legionella* after the chlorine level drops below 0.5 ppm
- Re-sample again after 2 weeks

Please note if there is a change of location for obtaining source water, a change of water conveyance, a power outage, a water main break, loss of biocide, corrosion, or conductivity control can cause conditions favorable to the multiplication of *Legionella*. Also note that using untreated recycled water as make-up water for cooling towers is also problematic. Whenever there is an increase in the concentration of *Legionella*, review your maintenance and treatment program. Chlorination is the most effective method for emergency cleaning but excessive chlorination will reduce the life of the system components. Deposits and biofilm reduce the efficacy of chlorination. Routine cleaning and chlorination will reduce the presence of *Legionella* but it is not permanent solution. The organisms will re-grow in a few weeks.
Unless you change your cooling tower maintenance and treatment program, *Legionella* will re-appear. An overall maintenance program should include routine shut down of the system to manually clean and flush the system components. Treatment should include the use of corrosion inhibitors, biodispersants (chemicals used to breakdown biofilm) and oxidizing and non-oxidizing biocides. Non-oxidizing biocide treatment will penetrate the biofilm accumulation on the tower components. Since *Legionella* multiply in the protozoans within the biofilm, controlling the biofilm is critical to controlling *Legionella*. The non-oxidizing biocides need to be rotated frequently to eliminate the development of resistant bacteria.

Improperly maintained hot tubs and whirlpool spas are increasingly being associated with legionellosis. The Association of Pool and Spa Professionals has comprehensive guidelines for maintenance that are available for purchase (www.apsp.org). Considerations for the proper maintenance of these features include the following: Single-use systems should be completely drained, cleaned between use and stored dry. Non single-use systems should be treated daily and cleaned weekly. These should be cleaned, treated and stored dry at the end of the season. Water treatment and filtration is essential whether these systems use potable or salt water. Heavy bather load will increase the need for cleaning, treatment, and filtration. Other organisms that can cause disease in these systems include *Non-tuberculosis Mycobacteria, Pseudomonas aeruginosa, Staphylococcus aureus, Naegleria fowleri* and *Cryptosporidium parvum* and *Giardia lamblia* and other organisms that are associated associated with fecal contamination caused by children in diapers.

In summary, all the remediation protocols mentioned here are for a short term, immediate response; they should not be considered as permanent solutions. If you return to operating your potable and non-potable water systems as before, the problem will return. Continuous, proper, routine maintenance and treatment is the only way to prevent the amplification of *Legionella*. 
EMSL Sampling Supplies

<table>
<thead>
<tr>
<th>EMSL Product Number</th>
<th>Description</th>
<th>Application</th>
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<tbody>
<tr>
<td>87M001</td>
<td>1000 mL Sterile Bottle w/ Preservative</td>
<td>Potable Water</td>
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<tr>
<td>87M005</td>
<td>250 mL Sterile Bottle</td>
<td>Non-Potable Water</td>
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<tr>
<td>8708321</td>
<td>Legionella Swab Sampling Kit</td>
<td>Biofilm or Slime</td>
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EMSL *Legionella* Test Codes

ISO Culturable Methods

<table>
<thead>
<tr>
<th>Test Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>M341 (M023)</td>
<td>Identification and Enumeration of <em>Legionella pneumophila</em> and other Legionella species (no species identification other than <em>L. pneumophila</em>)</td>
</tr>
<tr>
<td>M342 (M211)</td>
<td>Identification, Enumeration and Individual Serotyping of <em>Legionella pneumophila</em> serogroups 1 - 14 and 15 other Legionella species (no species identification other than <em>L. pneumophila</em>)</td>
</tr>
<tr>
<td>M343 (M212)</td>
<td>Identification, Enumeration and Individual Serotyping of <em>Legionella pneumophila</em> serogroups 1 - 14 and full species identification of 10 <em>Legionella</em> species (<em>anisa</em>, <em>bozemanii</em>, <em>dumoffii</em>, <em>gormanii</em>, <em>micdadei</em>, <em>longbeachae</em>, <em>jordanis</em>, <em>maceachernii</em>, <em>sainthelensi</em>, and <em>feelei.</em>)</td>
</tr>
<tr>
<td>M344</td>
<td>Identification, Enumeration and individual Serotyping of <em>Legionella pneumophila</em> serogroups 1 - 14 and full species identification of 10 Legionella species with 0 cfu/mL limit of detection (only for samples expected to have little to no bacteria present.)</td>
</tr>
<tr>
<td>M345</td>
<td>Identification, Enumeration and Serotyping of <em>Legionella pneumophila</em> serogroups 1 and 2-14 and other Legionella species (no species identification other than <em>L. pneumophila</em>)</td>
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</tbody>
</table>
## EMSL *Legionella* Test Codes

### CDC Culturable Methods

<table>
<thead>
<tr>
<th>Test Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>M210</td>
<td><strong>Level 1</strong> - Presumptive <em>Legionella</em> Detection – Presumptive enumeration</td>
</tr>
<tr>
<td>M023</td>
<td>Identification, Enumeration, and serotyping of <em>Legionella pneumophila</em> serogroups 1-14. Serogroup types are not enumerated individually.</td>
</tr>
<tr>
<td>M211</td>
<td><strong>Level 2</strong> - Identification, Enumeration, and individual serotyping of <em>L. pneumophila</em>, Serogroups 1-14</td>
</tr>
<tr>
<td>M213</td>
<td>Level 4 - CDC Heat Enrichment Plus Level 3 Identification for Samples Suspected of Containing High Levels of Protozoans</td>
</tr>
<tr>
<td>M214</td>
<td>Pure Culture Preparation and Storage</td>
</tr>
<tr>
<td>M215</td>
<td>Pure Culture Preparation and Shipping</td>
</tr>
</tbody>
</table>
# EMSL Legionella Test Codes

Polymerase Chain Reaction (PCR) *Legionella* Panels

<table>
<thead>
<tr>
<th>Test Code</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>M162</td>
<td>Presence/Absence of <em>L. pneumophila</em>, <em>L. micdadei</em>, <em>L. maceachernii</em>, <em>L. sainthelensis/cincinnatiensis</em></td>
</tr>
<tr>
<td>M103</td>
<td>Presence/Absence of <em>L. pneumophila</em></td>
</tr>
<tr>
<td>M102</td>
<td>Presence/Absence of <em>L. micdadei</em></td>
</tr>
<tr>
<td>M104</td>
<td>Presence/Absence of <em>L. sainthelensis/cincinnatiensis</em></td>
</tr>
<tr>
<td>M101</td>
<td>Presence/Absence of <em>L. maceachernii</em></td>
</tr>
<tr>
<td>M029</td>
<td>DNA Sequence Based Typing</td>
</tr>
</tbody>
</table>

*The Test Codes Below include M212 Level 3 culture, if Positive*
**EMSL Legionella Test Codes**

Test Code Selection Depending on Goal for Sampling

<table>
<thead>
<tr>
<th>Test Code</th>
<th>Goal for Sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>M210 or M023</td>
<td>Proactive Monitoring</td>
</tr>
<tr>
<td>M210 or M023</td>
<td>Determine Effectiveness of Maintenance Program</td>
</tr>
<tr>
<td>M212</td>
<td>Suspected Case</td>
</tr>
<tr>
<td>M211 or M212</td>
<td>Veterans Administration Hospital</td>
</tr>
<tr>
<td>M212</td>
<td>Hospital</td>
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<tr>
<td>M212</td>
<td>Nursing Home</td>
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<tr>
<td>M211</td>
<td>Confirmed Diagnosis of Legionnaires’ Disease Using Urinary Antigen Test</td>
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<tr>
<td>M213</td>
<td>Test includes heat enrichment if sample contains heavy biofilm, wastewater, sludge or potting soil</td>
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</tbody>
</table>

**Legionella Quick Tips**

**Potable Water**
- Collect from hot and cold water supplies if budget allows.
  - First draw - take 1 L directly from tap. Let water run for 60 seconds; take the second draw sample
- Aseptically remove aerators. Swab inside faucet or showerhead by circling the interior of the pipe 3 times.
- Sample every 6 months for routine monitoring.

**Non-Potable Water**
- Water from cooling towers, whirlpool spas, ornamental fountains – 250 mL - Inlet and outlet side; From any filters; Cooling tower pack and sump
- Leave air space in the bottle.
- Look for any slime or biofilm and take swab sample.
- Sample quarterly for routine monitoring.