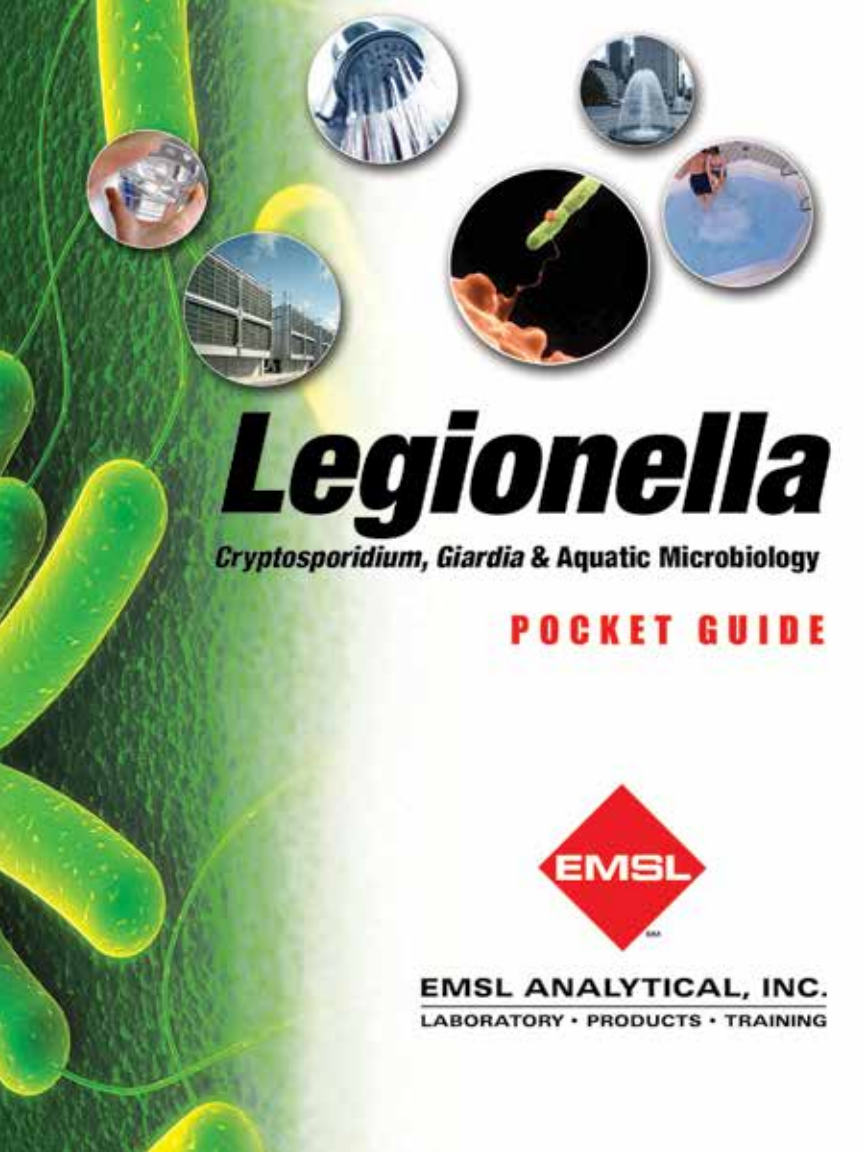

Legionella

Cryptosporidium, Giardia & Aquatic Microbiology

POCKET GUIDE

EMSL

EMSL ANALYTICAL, INC.

LABORATORY • PRODUCTS • TRAINING

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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 40 species of *Legionella* and 18 of those can cause disease. It is estimated 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 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. Legionnaires' Disease is most commonly linked to exposure to *Legionella pneumophila*. 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 risk factors for legionellosis are people who are immunocompromised by an underlying medical condition, those taking immunosuppresive drugs, heavy smokers, those who have chronic lung conditions, and the elderly. Several studies have documented 76 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

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. The American Society of Heating, Refrigeration and Air Conditioning Engineers (ASHRAE) recommends routine monitoring of building water supply systems.

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 links 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 Legionnnaires' 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.

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, compromised immune systems, and pre-existing medical conditions such as chronic obstructive pulmonary disease, cancer, and diabetes. 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. Pre-mature, 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 indicate 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.

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
10. Humidifiers/CPAP Machine Water Reservoirs
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

**Has not been associated with any cases of legionellosis to date but all the conditions exist for the presence and transmission of the bacteria.*

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 first.

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

Cooling Tower Sampling Locations (*Sample Quarterly)*

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

Potable Water Sampling Locations (*Sample Semi-Annually)*

- City Water Main Entry Point
- Hot Water Heater Drain Point*
- Storage Tanks
- 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 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:

Potable Water Sampling	Non-Potable Water Sampling
1 Liter Water Sample	*250 mL Water Sample*

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.

4. When sampling faucet aerators and showerheads, remove the aerators asceptically. 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, etc., collect 250 mL water from the bottom or side of the vessel or reservoir. If taking a cooling tower sample, also consider taking a sample in the pack column. 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 or on 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 lab. Phone: 800-220-3675, Fax: 856-786-0262, Email: info@emsl.com** *EMSL provides FREE sterile bottles and swabs for Legionella sampling (shipping cost to/from EMSL is not included).*

Emergency Remediation

Remediating *Legionella* after a case or an outbreak from potable and non-potable water is no easy matter and may require several attempts before the treatment is successful for achieving non-detectable results. 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.

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 tank and manually cleaning it of debris. Remove all deposits by scraping followed by wet vacuuming of the tank. through all the outlets including deadlegs and low use points and risers. Soak at this level

of free chlorine for 16 hours. Test to ensure that a minimum of 30 ppm free chlorine is obtained at all outlet 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 postive for *Legionella*, a major outtage, 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.*

Several studies have indicated that routine testing of a potable water system will identify a potential risk. Therefore, the goal is to establish a history of non-detectable results over time.

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; refill
- 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
- In both scenarios it is important to review your treatment program. There is a reason you got a positive *Legionella* result in the first place.

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 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 *Mycobacterium avium, Pseudomonas aeruginosa, Staphylococcus aureus, Naegleria fowleri* and organisms that are 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

EMSL Product Number	Description	Application
87M001	1000 mL Sterile Bottle w/ Preservative	Potable Water
87M005	250 mL Sterile Bottle	Non-Potable Water
8708301	Sterile Swab	Biofilm or Slime

EMSL *Legionella* Test Codes

CDC Culturable Methods (Recognized in US as Gold Standard)

Test Code	Description
M210	**Level 1** - Presumptive *Legionella* Detection – Presumptive enumeration
M023	Identification, Enumeration, and serotyping of *Legionella pneumophila* serogroups 1-14. Serogroup types are not enumerated individually.
M211	**Level 2** - Identification, Enumeration, and individual serotyping of *L. pneumophila*, Serogroups 1-14
M212	**Level 3** - Identification and Enumeration of *L. pneumophila plus L. anisa, L. bozmanii, L. dumoffii L. gormanii, L. jordanis, L. longbeachae, L. micdadei, L. macheachernii, L. sainthelensis. Individual serotyping included.*
M213	Level 4 - CDC Heat Enrichment Plus Level 3 Identification for Samples Suspected of Containing High Levels of Protozoans
M214	Pure Culture Preparation and Storage
M215	Pure Culture Preparation and Shipping

EMSL *Legionella* Test Codes

Polymerase Chain Reaction (PCR) *Legionella* Panels (Not Officially Recognized in US)

Test Code	Description
M163	Broad Screen *Legionella* Panel - Presence/absence of all 50 species of *Legionella* combined including *L. pneumophila.* Results include: *L. pneumophila:* 16S rRNA gene and mip gene; *L. anisa, L. oakridgensis, L. bozemanii, L. birminghamensis, L. brunensis, L. cherri, L. cincinnatiensis, L. dumoffii, L. erythra, L. fairfieldensis, L. feelei, L. gormanii, L. gratiana, L. hackeliae, L. israelensis, L. jamestownensis. L. jordanis, L. lansingensis, L. longbeachae, L. maceachernii, L. micdadei, L. moravica, L. parisiensis, L. quinlivanii, L. rubrilucens, L. santicrucis, L. sainthelensis, L. spiritensis, L. steigerwaltii, L. tucsonensis. L. wadsworthii, L. worsliensis*
The Test Codes Below include M212 Level 3 culture, if Positive*	
M162	Presence/Absence of *L. pneumophila, L. micdadei, L. maceachernii, L. sainthelensis/cincinnatiensis*
M103	Presence/Absence of *L. pneumophila*
M102	Presence/Absence of *L. micdadei*
M104	Presence/Absence of *L. sainthelensis/cincinnatiensis*
M101	Presence/Absence of *L. maceachernii*
***Legionella pneumophila* serotype 1 Strain Identification**	
M029	DNA Sequence Based Typing

EMSL *Legionella* Test Codes

Test Code Selection Depending on Goal for Sampling

Test Code	Goal for Sampling
M210 or M023	Proactive Monitoring
M210 or M023	Determine Effectiveness of Maintenance Program
M212	Suspected Case
M211 or M212	Veterans Administration Hospital
M212	Hospital
M212	Nursing Home
M211	Confirmed Diagnosis of Legionnaires' Disease Using Urinary Antigen Test

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.

What Are *Cryptosporidium* and *Giardia*

Cryptosporidium and *Giardia* are waterborne pathogens which cause the intestinal illnesses cryptosporidiosis and giardiasis, respectively. Symptoms of infection include watery diarrhea, stomach cramps or pain, nausea, dehydration, vomiting, fever, and weight loss. These diseases are generally transmitted by eating contaminated food, drinking contaminated water, and swallowing contaminated water while swimming or bathing.

Cryptosporidium Overview

Cryptosporidium is commonly known as "Crypto" and was first described by E. E. Tyzzer in 1907. There are many species of *Cryptosporidium* that infect humans and animals. The parasite is protected by an outer shell that allows it to survive outside the body for long periods of time and makes it very tolerant to chlorine disinfection. While this parasite can be spread in several different ways, water (drinking water and recreational water) is the most common method of transmission.

Cryptosporidium is one of the most frequent causes of waterborne disease among humans in the United States. The 1993 Milwaukee *Cryptosporidiosis* outbreak was the largest waterborne disease outbreak in documented United States history. Over the span of approximately two weeks, 403,000 of an estimated 1.61 million residents in the Milwaukee area became ill. At least 104 deaths have been attributed to this outbreak, mostly among the elderly and immunocompromised people, such as AIDS patients.

Epidemiology

Cryptosporidium lives in the intestine of infected humans or animals. Millions of Crypto germs can be released in a bowel movement from an infected human or animal. Shedding of Crypto in the stool begins when the symptoms begin and can last for weeks after the symptoms (e.g., diarrhea) stop. People can become infected after accidentally swallowing the parasite. Crypto is not spread by contact with blood.

People with greater exposure to contaminated materials are more at risk for infection, such as:

- Children who attend day care centers, including diaper-aged children
- Child care workers
- Parents of infected children
- People who take care of other people with cryptosporidiosis

- International travelers
- Backpackers, hikers, and campers who drink unfiltered, untreated water
- People who drink from untreated shallow, unprotected wells
- People, including swimmers, who swallow water from contaminated sources
- People who handle infected cattle
- People exposed to human feces through sexual contact

Monitoring Guidelines

Long Term 2 Enhanced Surface Water Treatment Rule (LT2 Rule) was published in the Federal Register on January 5, 2006 to reduce disease incidence associated with Crypto and other disease-causing microorganisms in drinking water. The rule applies to all systems that use surface water or grounder water under the direct influence of surface water.

An Overview of *Cryptosporidium* and *Giardia*

EPA initiated an effort in 1996 to identify new and innovative technologies for protozoan monitoring and analysis. The EPA Method 1623 to detect *Cryptosporidium* and *Giardia* was therefore initially released in April 1999 (EPA-821-R-99-006) and lastly revised in 2005 (EPA-821-R-05-002). The Method 1623 was then revised again in January 2012 to become Method 1623.1 (EPA 816-R-12-001). Both 1623 and 1623.1 are acceptable methods to analyze *Cryptosporidium* and *Giardia* in the LT2 Rule. The major procedures in the methods include filtration, immunomagnetic separation (IMS), and immunofluorescence assay (FA) microscopy. *Cryptosporidium* and *Giardia* are further characterized using 4',6-diamidino-2-phenylindole (DAPI) staining and differential interference contrast (DIC) microscopy.

EMSL has been certified in 35 states to analyze *Cryptosporidium* and *Giardia* using EPA methods.

Cryptosporidium and *Giardia* samples

EMSL accepts both the bulk water samples (10 liters) and field filtered samples (10 or 50 liters). EMSL provides the sampling kits for bulk water samples and field filtration apparatus rental (or purchase) for field filtered samples. A matrix spike sample must be submitted at the beginning of the monitoring and every 20 samples afterwards for the same water source.

Results are normally provided within 2 weeks. Rush analysis as quick as 3 days is available with additional charges. Please read the following for the detailed sampling and shipping information.

Sampling Instructions
Cryptosporidium and *Giardia*
Instructions for Field Filtered Samples (10 or 50 Liters)

EMSL Field Filter Sampling Kit includes

- 1 Shipping container (Cooler)
- 2 Plastic trash bags
- 2 Temperature labels (Freeze™ and WarmMark®) or 1 Temperature logger
- 1 Pair of gloves
- 1 Chain of Custody (COC)
- Envirochek™ filter capsule(s)
- 1 Plastic sample bag (Ziploc style) for each Envirochek™ filter capsule(s)
- Bubble wrap
- Refrigerant packs

EMSL Field Filtration Unit (optional) includes

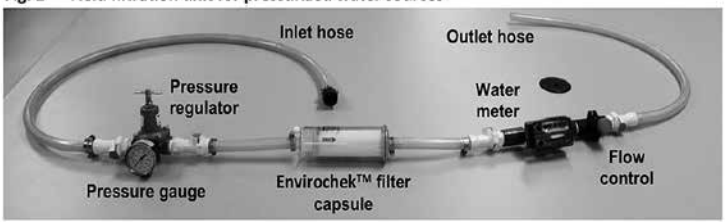
- Inlet and outlet hoses
- Pressure regulator with pressure gauge
- Water meter (flow rate and totalizer)
- Flow control valve
- Pump (optional, for unpressurized water source only)
- Clamps

Items that are required and not provided include

- Waterproof pen
- Turbidimeter

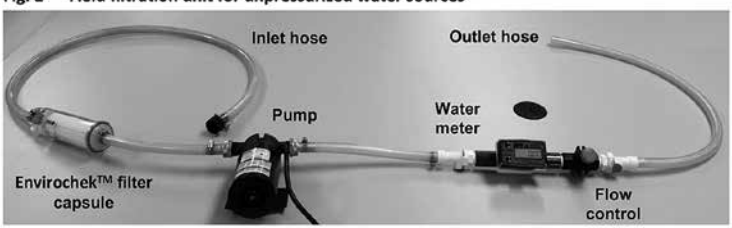
1. <u>For pressurized water sources,</u> connect the field filtration unit (Fig. 1), without the Envirochek™ filter, to the water source. Adjust the pressure to below 60 PSI using pressure regulator and to a flow rate no greater than 2.0 L/min (0.5 G/min) using flow control knob. Flush the sampling unit for 2-3 minutes (or 2- 10 L of sample water). The water meter functions as both a totalizer and flow rate meter. Alternate functions by pressing the "DISPLAY" button. Select the desired units (gallon (GL), liter (LT), or cubic foot (CF)) for the totalizer by pressing "DISPLAY" when "CALIBRATION" is pressed.

Fig. 1 Field filtration unit for pressurized water sources



2. <u>For unpressurized water sources,</u> connect the field filtration unit (Fig. 2), without the Envirochek™ filter, to a carboy with 30+ L water. Adjust the flow rate to no greater than 2.0 L/min (0.5 G/min) using flow control knob. Flush the sampling unit for 2-3 minutes (or 2- 10 L of sample water).

Fig. 2 Field filtration unit for unpressurized water sources



3. Insert and connect the filter capsule into the filtration unit. Ensure the directional flow arrow is pointing toward the water meter. Use clamps to secure the inlet and outlet if needed.

4. Record date, time, initial/final flow totalizer reading, sample ID, sample turbidity, sampling location, and the Operator's name on the label of the filter using a water-proof pen.

5. Keep filter capsule vertical. Vent residual air by gently tapping the capsule or using the bleed valve/vent port, if necessary.

6. Filter 10 L (2.64 Gal) or 50 L (13.21 Gal) of water sample. Record stop time and final flow totalizer reading.

7. Hold inlet end of filter capsule pointing up and allow water to drain. Open bleed valve/vent port to speed the draining process, if needed.

8. Replace the blue vinyl caps on each end of the filter.

9. Seal each filter in a separate Ziploc style bag.

10. Refrigerate each filter as soon as possible and store under refrigeration (1-10°C) until shipment.

Instructions for Matrix Spike (MS) Samples

1. MS samples must be collected every 20 routine field filtered samples and for each water source. The MS sample and routine field filtered sample must be collected from the same location at the same time. The collected MS sample volume must be within 10% of the collected routine field filtered sample volume.

2. For a 10 L MS sample, collect one bulk water sample according to the instructions on page 20.

3. For a 50 L MS sample, filter all but 10 L of the MS sample at your sampling location and collect the remaining 10 L as a bulk water sample. Clearly label both the bulk water and the filter sample as comprising 2 parts of a single sample.

4. Refrigerate your bulk water and filter samples as soon as possible and store under refrigeration (1-10°C) until shipment.

Cleaning procedure between samples

1. Thoroughly clean inlet hose and pressure regulator/gauge (for pressured sources only) with a warm detergent solution.

2. Expose to hypochlorite solution (25 mL of 5% household bleach for every 1 gallon of pH 7 water) for at least 30 minutes at room temperature.

3. Thoroughly rinse with reagent grade water that is free of Crypto/Giardia. Dry in an area free of Crypto/Giardia.

Packing and Shipping

1. Insert two large plastic trash bags into the cooler to create a double liner.

2. Apply Freeze™ and WarmMark® labels to the clean and dry outside surface of each Envirochek™ filter and activate the WarmMark® label by folding up and pulling the upper portion.

3. Place each Envirochek™ filter, with temperature labels affixed, inside its own, sealed, Ziploc bag.

4. Wrap the sealed, bagged filter sample(s) in the bubble wrap provided and place the wrapped samples into the shipping container (cooler) lined with the two large plastic trash bags.

5. If a temperature logger is provided rather than the temperature labels, wrap the logger in bubble wrap and place it immediately next to the filter sample(s) within the shipping container (cooler).

6. Place sufficient refrigerant packs and/or ice bags (sealed in Ziploc bags or equivalent) around bubble wrapped filter(s) to maintain a sample temperature range of 1-20°C during shipment. NEVER USE DRY ICE.

7. Knot and seal the two large plastic bags. Place completed Chain of Custody form (in sealed Ziploc style bag) on top of knotted plastic bags. Seal the cooler for shipping.

8. Refer to instructions on page 20 to pack and ship the 10 L bulk water sample as MS sample.

9. Ship all samples and sampling kits via OVERNIGHT delivery to: **Attn: Aquatic Microbiology, EMSL Analytical, Inc., 200 Route 130 North, Cinnaminson, NJ 08077 Phone: 800-220-3675, Fax: 856-786-0262, Email: info@emsl.com**

Sampling Instructions
Cryptosporidium and Giardia
Instructions for 10 Liter Bulk Water Samples

EMSL Bulk Water Sampling Kit includes

- 1 Shipping container (Cooler)
- 2 Plastic trash bags
- 2 Temperature labels (Freeze™ and WarmMark®) or 1 Temperature logger
- 1 Cubitainer (10 L)
- 1 Pair of gloves
- 1 Chain of Custody (COC)

Items that are required and not provided include

- Refrigerant packs or ice bags
- Waterproof pen

Sampling

1. Fill the 10 L cubitainer to the neck and store under refrigeration (1-10°C) until shipment. If samples are collected later in the day, these samples may be chilled overnight in a refrigerator (1-10°C).

2. Indicate sampling date, time, location, and name of sampler on the cubitainer using waterproof pen.

Instructions for Matrix Spike (MS) Samples

1. MS samples must be collected every 20 routine bulk water samples and for each water source. The MS sample and routine bulk water sample must be collected from the same location at the same time. The collected MS sample volume must be within 10% of the collected routine bulk water sample volume.

2. For a 10 L MS sample, collect one bulk water sample according to the previous instructions.

3. Refrigerate your MS sample as soon as possible and store under refrigeration (1-10°C) until shipment.

Packing and Shipping

1. Before shipment, insert two large plastic trash bags into the cooler to create a double liner.

2. Place the chilled bulk water sample inside the double lined trash bags.

3. Apply Freeze™ and WarmMark® labels to the clean and dry outside surface of the cubitainer, and activate the WarmMark® label by folding up and pulling the upper portion. If a temperature logger is provided rather than the temperature labels, place it immediately next to the bulk water sample (inside of the trash bags).

4. Knot and seal the innermost plastic trash bag containing the bulk water sample (with temperature labels affixed) and/or the temperature logger.

5. Add sufficient SEALED ice bags or refrigerant packs around the sealed sample and inside the second, outermost plastic trash bag to maintain a sample temperature range of 1-20°C during shipment. NEVER USE DRY ICE.

6. Knot and seal the outermost second plastic trash bag. Place completed Chain of Custody form (in sealed Ziploc style bag) on top of knotted plastic bags. Seal the cooler for shipping.

7. Ship all samples and sampling kits via OVERNIGHT delivery to: **Attn: Aquatic Microbiology, EMSL Analytical, Inc., 200 Route 130 North, Cinnaminson, NJ 08077 Phone: 800-220-3675, Fax: 856-786-0262, Email: info@emsl.com**

Important Notes

- Samples will be rejected if sample temperature upon laboratory receipt is >20°C or frozen per EPA method.
- Please schedule sample collection to allow samples to be received at the laboratory Monday through Thursday.
- Please contact EMSL with any sampling and/or shipping questions.

Sampling Instructions
Microscopic Particulate Analysis (MPA)

EMSL Sampling kit includes:

- Inlet hose with backflow preventor
- Pressure regulator and gauge
- LT-10 filter housing (9499-5015)
- Cartridge filter (s) (M39R10A)
- Water meter (flow rate meter and totalizer)
- Flow control valve
- Discharge hose
- Pump (optional, for non-pressurized water sources)
- Proportionating injector (optional, for chlorinated water only)
- Clamps for unit assembly
- Whirlpak plastic bags or zip loc heavy duty quality freezer bags
- Gloves
- Chain of Custody (COC)

Sampling Parameters

Consensus Method

(Groundwater Under the Direct Influence of Surface Water (GWDI)) Avoid sample sites within the distributed system. Sample(s) should be collected prior to any blending, disinfection or other treatment. Minimum 500 gallons, recommend 1,000 gallons over an 8–24 hour period per EAP requirement.

Filtration Plant

Raw Surface Water

- Sampling prior to chemical addition and after any presedimentation basins (if no chemicals were added prior to presedimentation). If recycling operations are practiced, the raw water should be sampled after the recycling input.

- A minimum volume of 100 liters (27 gallons) for a 12 to 24 hour period. The ideal volume is the amount equivalent to a complete day of production. If the filter becomes clogged or plugged due to highly turbid waters, terminate sampling and record the volume collected to this point.

Finished Water

- Sampling after the filtration system and prior to chlorine addition, if possible.
- Minimum 1000 liters (264 gallons). Collection period should encompass a full cycle run, or for 24 hours, including at least one backwash cycle. Backwash cycles can occur at the initiation of the sampling period.

Chlorinated Samples

If chlorinated water must be sampled, an injector system will need to be installed to add a sodium thiosulfate solution to denature the chlorine. Add sodium thio sulfate solution via the injector system to produce a final concentration of 50 mg/L. Setting the injector system to produce a 1:100 dilution of 0.5% sodium thiosulfate stock solution will result in a final concentration of 50 mg/L.

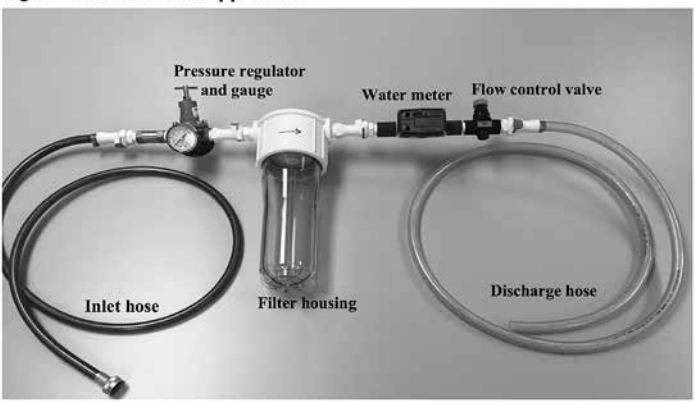
Treatment Plant Evaluation

The raw water sampling should be initiated before the finished water sampling. The amount of time elapsed between the beginning of raw sampling and the beginning of finished sampling should be equivalent to the detention time of the system.

Sampling - For Pressurized Water Sources

1. Connect the field filtration unit (Fig. 3), without the cartridge filter, to the water source. Flush the unit for 3 - 5 minutes with the source water to be sampled. If collecting samples from multiple locations, run a minimum of 50 gallons of sample water through the sampling equipment between samples to avoid cross contamination. Adjust the flow rate to 1 gpm (3.8 lpm) using flow control valve. Adjust pressure gauge to 10 psi using pressure regulator. Adjust both flow control valve and pressure gauge so that 1gpm flow rate and 10psi pressure are maintained. The water meter functions as both a totalizer and flow rate meter. Alternate functions by pressing the "DISPLAY" button. Select the desired units (gallon (GL), liter (LT), or cubic foot (CF)) for the totalizer by pressing "DISPLAY" when "CALIBRATION" is pressed at the same time.

Fig. 3 MPA filtration apparatus



Sampling - For Non-Pressurized Water Sources

1. Add a pump between filter and water meter. Be sure to put the sample intake in a location where the least amount of bottom sediment will enter into the sampling filter giving a distorted view of the sample. Adjust flow rate and pressure gauge as in Section 3.1. Note: Collect sample as near to intake site as possible.

2. Insert filter into the housing and hand tighten housing. Make sure provided clamps are in place and tight at both inlet and outlet of the housing. Do not touch the filter with bare hands, use sanitary gloves or the plastic cover the filter is wrapped in. Turn water on slowly with the unit in an upright position. Invert unit to make sure all the air within the housing is expelled. Record the date, time of day, and gallon reading from the water meter before sampling.

3. When the filter housing is full of water, return unit to upright position, maintain the flow rate at 1 gpm (3.8 lpm) and pressure at 10 psi throughout the sampling period.

4. The sampling unit should be allowed to run for an 8-24 hour period according to the sampling parameters in Section 2.0.

5. After filtering sample turn off the faucet or pump and disconnect the hose from incoming water source. Maintain the inlet hose level above level of opening on outlet hose to prevent backwashing and loss of particulate matter from the filter. Pour residual water from filter housing into a Ziploc bag.

6. Remove the sampling cartridge with the plastic cover or sanitary latex gloves. Do not touch with bare hands. Place filter in a second heavy duty quality ziploc (whirlpak) bag and seal.

7. Record stop time and final meter reading. Subtract the initial reading from the final reading and record the total volume collected. Document the name and location of each sample point, sampling site (raw or finished) and type of treatment.

8. With permanent marker record the sample identification, gallons sampled, collection dates and times, collector's name and water quality parameters directly on the bag or on a waterproof label. Double pack the bags containing filter and residual water with ziploc bags. Make sure all bags are sealed to prevent leakage.

9 . If immediate shipping is not possible, the sample should be stored in a 1-5°C refrigerator, until shipping.

10. Place freezer cold packs in the shipping container. Place insulating material between the filter and cold packs to prevent sample freezing. Samples that arrive at the laboratory frozen will be rejected. Place data sheet containing recorded information in a sealed plastic bag and ship with the filters.

11. Ship all samples and sampling kits via OVERNIGHT delivery to: **Attn: Aquatic Microbiology, EMSL Analytical, Inc., 200 Route 130 North, Cinnaminson, NJ 08077 Phone: 800-220-3675, Fax: 856-786-0262, Email: info@emsl.com**

EMSL Aquatic Microbiology Test Codes

Test Code	Description
M600	Phytoplankton identification (division level)
M601	Phytoplankton identification (genus level)
M602	Phytoplankton identification (species level)
M603	Phytoplankton identification and enumeration (genus level)
M604	Phytoplankton identification and enumeration (species level)
M605	Phytoplankton identification, enumeration, and biovolume (genus level)
M606	Phytoplankton identification, enumeration, and biovolume (species level)
M607	Periphyton (attached algae) identification and enumeration (division level)
M608	Periphyton (attached algae) identification and enumeration (genus level)
M609	Periphyton (attached algae) identification and enumeration (species level)
M610	Algae permanent slide HPMA (3 slides per sample)
M611	Algae permanent slide Naprax diatom (3 slides per sample)
M612	Biomass-Dry weight
M613	Biomass-Ash-free dry weight
M614	Chlorophyll *a*
M615	Chlorophyll *a* & Pheophytin *a*
M616	Chlorophyll *a, b, c* and Pheophytin *a*
M617	Taste and Odor Test (Geosmin and 2-MIB)
M618	Microcystin
M619	Cylindrospermopsin

EMSL Aquatic Microbiology Test Codes

Test Code	Description
M620	Saxitoxin
M630	*Cryptosporidium* and *Giardia* (EPA 1623)
M631	*Cryptosporidium* (EPA 1623)
M632	*Giardia* (EPA 1623)
M638	*Cryptosporidium* (EPA 1623.1)
M639	*Giardia* (EPA 1623.1)
M640	*Cryptosporidium* and *Giardia* (EPA 1623.1)
M641	Matrix Spike for *Crypto* & *Giardia* testing
M642	Additional filter analysis for *Crypto* & *Giardia* testing
M643	Additional subsamples for *Crypto* & *Giardia* testing
M644	Bacterial abundance (DAPI or AO)
M645	Zooplankton identification to major taxa
M646	Zooplankton total count (major taxa)
M647	Zooplankton identification and enumeration (major taxa)
M648	Zooplankton identification and enumeration (Genus)
M649	Zooplankton identification and enumeration (Species)
M650	Zebra Mussel Veligers count
M651	Permanent slide for zooplankton
M652	Invertebrates Identification to major taxa
M653	Microscopic Particulate Analysis (MPA)

EMSL ANALYTICAL, INC.

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