Environmental Sampling for *Legionella* Bacteria

SCOPE

This guideline sets out the procedures for the collection of environmental samples to be examined for the presence of *Legionella* bacteria when investigating the source of infection in cases of legionellosis. It details sampling from the following sources:

- Reticulated hot and cold water systems, including bulk storage tanks (see Section A, page 4)
- Spa pools (see Section B, page 7)
- Cooling towers and evaporative condensers (see Section C, page 8)
- Compost, potting mixes, and soil (see Section D, page 8)
- Natural pools, thermal baths & decorative fountains (see Section E, page 9)

Prior to collecting environmental samples from any site, please notify the Environmental Microbiology Laboratory (EML) of your intentions. EML will provide appropriate sample containers for the collection and transport of all samples to the laboratory. Please contact the EML directly for sample containers.

For any suspected sites not listed above, please contact the EML for instructions.

Contact details for EML can be found on page 11 at the end of this guideline.

For details concerning the packaging shipping of samples, refer to page 11.

INTRODUCTION

Legionellosis is a notifiable disease. Infection with *Legionella* bacteria can cause a spectrum of disease, but is commonly divided into two distinct clinical syndromes, grouped together under the name legionellosis. The first is Pontiac fever, a self-limiting influenza-like illness with no radiological evidence of pneumonia and usually with a 24-48 hour incubation period. Patients recover spontaneously in 2-5 days. The second is Legionnaires' disease, a severe and potentially fatal form of pneumonia, generally with a 2-14 day incubation period and requiring medical intervention. Legionellosis frequently occurs in clusters and has the potential to cause explosive outbreaks. There is no human-to-human transmission, with all cases having to be exposed to a contaminated environmental source. Source tracing of cases is an important aspect of any outbreak control and disease surveillance programme.

APPLICATION

The Environmental Microbiology Laboratory (EML) at ESR carries out testing for the presence of *Legionella* bacteria in environmental samples. The laboratory holds IANZ accreditation for the methods used for the isolation of *Legionella* from environmental samples. Any *Legionella* bacterium isolated is identified to the species and serogroup level in conjunction with the Legionella Reference Laboratory at ESR.

SAMPLING OBJECTIVES

The objectives of environmental sampling are as follows:

- Confirmation or exclusion of an implicated site as the source of infection
- Risk assessment of a reticulated water system
- Distinguishing between localised or system-wide colonisation of the water system
- Establishing the efficacy of any disinfection strategy for a contaminated water system
- Selecting the most appropriate strategy for both the short term and long term control of *Legionella* at the site
- Risk assessment of a composting facility

ECOLOGY OF *LEGIONELLA*

Understanding aspects of *Legionella* ecology will help identify potentially contaminated sites that pose an environmental risk. *Legionella*e are ubiquitous microorganisms that live as either intracellular parasites of protozoa in aquatic environments and soils, as biofilm-bound sessile forms or as free-living planktonic forms. From natural sources such as rivers, lakes and reservoirs, the organism can enter reticulated water systems.

Water temperatures in the range 20°C to 50°C favour growth of *Legionella*, with their optimum temperature range between 30°C and 45°C. *Legionella* will not replicate at temperatures above 50°C and are rapidly killed at temperatures above 60°C. *Legionella* remains dormant at temperatures below 20°C but multiply when the temperature reaches a more suitable level.

The growth and persistence of *Legionella* is strongly influenced by commonly encountered organisms within water systems, such as algae, protozoa and other bacteria. The presence of sediment, sludge, scale and other material within the system, together with surface biofilms, also provide favourable conditions for harbouring *Legionella* and allowing it to proliferate. *Legionella* requires an essential amino acid, *L*-cysteine, for grow and this must be provided from an external source, i.e., other microorganisms. Many disinfection processes used for the control of *Legionella* in water systems, such as sodium hypochlorite treatment or UV radiation, penetrate poorly into biofilms allowing *Legionella* to escape.

As an intracellular facultative parasite, *Legionella* is able to invade and multiply within some protozoan species. When the protozoan host encysts because of unfavourable environmental conditions the *Legionella* remains viable but dormant. This may be another reason why *Legionella*e are shielded from the effects of biocides and can persist within water systems following disinfection. *Legionella* has also been shown to multiply in the gut of the nematode *Caenorhabditis elgans.* This may partly explain the prevalence and persistence of *Legionella* in composted material as the nematode is found in composting vegetative material.

CHOOSING APPROPRIATE SAMPLING SITES

Sampling sites should be chosen to be representative of all the identified risk areas where *Legionella*e can reside and grow. The approach taken for choosing sampling sites is generally dependent on the nature of the site. For large sites this may necessitate taking multiple samples. To begin with examine sites to establish all systems using water or compost, considering the following:

- Areas which contain water at temperatures likely to support the growth of *Legionella* (i.e. above 20°C but below 55°C)
- Pipe work where there is cross-contamination between “dead” (stagnant water or slow-moving water) and “live” water (i.e. presence of ‘deadlegs’)
- Domestic water reticulation systems where the source water is not treated, e.g. where the source water is roof-collected rain water or bore water
- Locations where water aerosols can be created and released into the atmosphere
- Sites containing composted vegetation or the potential to create aerosolised dust from this material

All water storage tanks, hot water cylinders, decorative fountains, spa baths, spa pools, thermal pools, misting machines, water spraying devises, water blasters, cooling towers, humidifiers, any water system with either a recirculating water system or water reservoirs where water can reach temperatures greater than 20 C but less than 55 C must be considered potential sources for the growth of *Legionella* bacteria.

All of the systems or items listed above must be seriously considered for the taking of environmental samples for *Legionella* bacteria culture when investigating suspected and confirmed cases of legionellosis.

Reticulated water systems often become contaminated with *Legionella* from the deposition of organic matter carried into an open system. This may occur from wind-blown dirt into an exposed reservoir such as a cooling tower. Contamination of reticulated water systems can occur during construction activity or alterations to a building and where earth works is carried out. Another potential cause for *Legionella* contamination of reticulated water systems is through soil contamination during plumbing work involving soil excavations. When investigating potential sources of infection, determine if any recent plumbing work has been carried out at the site or in the immediate water reticulation system. The occurrence of leaks or breaks in water pipes and also their subsequent repair can be origin for introducing *Legionella* into the water reticulation system.

Another major recognised source of *Legionella* bacteria infection in New Zealand is compost and materials containing compost, such as potting mixes, seed-raising mixes, and garden mulches – including bark mulch, etc. The use of this type of material, or close exposure to it in the 10 to 14-day incubation period prior to the onset of disease symptoms implicates it as a potential infection source.

With judicious questioning of the case and by carefully inspecting potential exposure sites frequented by the case during their disease incubation period, the most likely exposure sites can be narrowed down to minimise the amount of environmental sampling required. With all risk sites being identified, the appropriate samples for *Legionella* culture can be made.

SAFETY CONSIDERATIONS WHEN SAMPLING

The mode of transmission for legionellosis is by the inhalation of either dust or water aerosols containing *Legionellae*. The aspiration of water contaminated with *Legionella* has also been described as a mode of transmission. Personnel taking samples where a water system is implicated as the source of infection should observe all the correct occupational safety and health procedures. Precautions should be taken to avoid exposure to any aerosols generated during sampling. Even taps run gently will create aerosols. Be aware that when sampling water or compost there is a risk of being exposed to water or dust aerosols. As a minimum precaution wear a correctly fitting half-face respirator with a HEPA filter (for example a N99 respirator) at all times while sampling to minimise exposure to aerosols.

In all cases of sampling, hands should be washed and thoroughly dried after sampling and before eating, drinking or smoking. Personnel carrying out sampling should have received training in risk assessment and the control of *Legionella*.

Personnel should be trained in the correct use of ladders. Personnel should be aware of the dangers to prevent falls when moving in ceiling spaces. Protective clothing and dust masks should be worn to avoid contact with or inhalation of insulation material when working in ceiling spaces.

EQUIPMENT AND MATERIALS

The items listed below are required for collecting samples from environmental sources:

Sampling Equipment:

- Sterile 1-litre capacity sample containers containing 200 mg of sodium thiosulphate to neutralise any chlorine or other halogen-based biocides
- Plastic bags – food grade, new
- Scissors
- Sterile metal dip sampler or similar
- Sterile swabs and containers
- Timer
- Thermometer – calibrated and able to measure between 10°C & 100°C
- Plastic wash bottle containing 1:10 dilution of sodium hypochlorite (bleach) *or* neat methylated spirits *or* 80% ethanol *or* 80% isopropanol
- Alcohol wipes
- Paper towels
- Chilly bins (for placing samples after collection)
- Zip-lock sterile plastic bags

Personal protective equipment:

- Disposable latex gloves
- Overalls and sturdy footwear
- N99 HEPA filter ½ face respirator or similar (P2 face respirator)

Other optional equipment that can be found useful:

- Ladder
- Torch and batteries
- Assorted spanners and screwdrivers
- Scalpel blade or similar (useful to detach the plastic lug covering the screw head in the centre of some shower roses)

A. SAMPLING FROM RETICULATED WATER SYSTEMS

A.1 Establish plan of hot and cold water systems

On entering the property carry out a preliminary survey to establish the layout of the hot and cold water reticulation system. Establish the positions of any header tanks, water heaters and water storage cisterns. Establish the positions of all hot water outlets (taps, shower heads, etc.) and which water heater and which header tank that they are connected to.

Carry out the same procedure for the cold water system. Establish the position of header tanks, bulk cold water storage tanks and water storage cisterns. Establish the positions of all cold water outlets (taps, shower heads, toilets, etc.) and which header tank that they are connected to. In all situations, identify the outlets were used by the case and ensure samples are collected from them. *The survey is done to indicate the most appropriate places from where samples should be collected.*

A.2 Physical inspection

Inspect the piping. Note the type of materials used for the piping and outlets (copper, galvanised steel, PVC, etc.). Inspect the water heater(s). Note the type of heater: whether electrical immersion or gas, and whether instant or storage. Record the water temperature setting on the thermostat and establish how frequently the water heater is turned off and on.

Low temperature settings, and/or a history of frequently turning the water heater off increase the likelihood of Legionella *contamination. Instant gas water heaters fed from a treated potable water supply with an ambient water temperature below 20°C are a low risk source for* Legionella *contamination.*

A.3 Sampling procedure

A.3.1 General considerations

Samples taken from reticulated water systems consist of swabs (biofilm) and water samples. Samples need to be taken from various points in the water reticulation system where *Legionella* can grow. Collect water samples representing the bulk water in any storage tank as well as the pipe work of any warm water reservoir. Generally water samples need to be taken from the water heater, the outlet closest to the water heater and the outlet most distal to the water heater.

Ideally, swab (biofilm) samples must be taken before water samples when collecting both sample types from the same outlet. This allows capture of any dislodged biofilm not caught on the swab. Swab (biofilm) samples are collected from the interior of any outlet where water is aerosolised, such as showers and misters. Usually, it is not possible (and therefore no practical reason) to collect swab samples from simple tap outlets as there is limited access to wet piping and the part of the outlet that is accessible is usually dry.

Water samples are either “First Catch” or “Second Catch” samples. The “First Catch” sample represents water immediately adjacent to the outlet and captures microflora at the outlet and in the adjacent piping, while the “Second Catch” sample represents water from the source supply (or storage vessel) and captures microflora resident there.

For any reticulation system where water is stored, a single “Second Catch” sample must be collected from each storage vessel. Any “Second Catch” sample must be taken from the water outlet closest to the water storage tank if direct sampling from the storage tank is not possible. If a “First Catch” sample is also to be collected from an outlet from which a “Second Catch” sample is also being collected, then water must be run to waste between collecting the two samples. The time spent running water to waste between collecting the “First Catch” sample and the “Second Catch” sample will depend on the length of the piping between the source tank and the outlet being used as well as the water flow rate used. For most domestic situations a 2-minute ‘flush’ period when running water from the outlet with the tap or valve fully open is usually sufficient to remove all ‘hold-up’ water in the pipe work.

Some hot water heaters have a tempering valve fitted to the hot water outlet of the water heater. This device is used to control the temperature of the hot water leaving the heater by introducing cold mains water into the heated water resulting in the hot water being cooled and also reducing the effect of any thermal disinfection of the water resident in the water heater tank.

A.3.2 Hot water outlet nearest to the water heater

- **Inspection:** Record if the outlet has any defects (dripping, leaking washers, etc.). Note where the outlet is and its type (single tap, shower, mixer tap, etc.).
- **Sampling:** If this is a shower, then follow the procedures below for a Shower (A.3.4). If it is a single tap, then follow the procedures below for a Tap outlet (A.3.5). Water samples from the hot water outlet nearest to the water heater consist of a "First Catch" water sample, and a "Second Catch" water sample. Ensure sample containers are labelled clearly to distinguish between the "First Catch" and "Second Catch" samples.
- **Temperature measurement:** Take the temperature of the water at the outlet after the samples have been taken and after the water has flowed for at least 2 minutes.

A.3.3 Hot water outlet most distal to the hot water heater

- **Inspection:** Record if the outlet has any defects (dripping, leaking glands, etc.). Note where the outlet is and its type (single tap, shower, mixer tap, etc.).
- **Sampling:** Collect a one litre "First Catch" sample from the hot water outlet furthest from the hot water heater. Use the same sampling procedure as for the nearest hot water outlet.
- **Temperature measurement:** Take the temperature of the water at the outlet after the samples have been taken and after the water has flowed for at least 2 minutes. Disinfect the thermometer after each use with an alcohol wipe. Change gloves before the next sample.

A.3.4 Showers

- **Inspection:** Inspect the shower noting its type, whether it has a mixer or direct feed from the hot and cold supplies, whether it has a flexible hose or not and if it has any faults including dripping, scale build-up, slime growth or deterioration of the hose.
- **Swab (biofilm) sample:** Take the swab sample before taking any water samples from a shower. To take the swab sample, first remove the showerhead. Label a swab sample container and remove the sterile swab from the sample container. Moisten the swab cotton tip with a little shower water. Collect a swab sample from the interior of the pipe by vigorously rubbing the surface of the pipe for as far as the swab can reach. Collect the biofilm sample from as much of the inner surface as possible. Replace the swab into its sleeve with 1.0-2.0 mL of the same residual water to prevent the swab from drying out. Reassemble the showerhead before proceeding.
- **"First Catch" sample:** Label a sample container, then remove its lid, storing it in a clean new plastic bag to prevent contamination. Cut the bottom corner off a new food grade plastic bag. Insert the shower rose into the bag and hold the bag securely enclosing the shower rose. Insert the cut-off corner of the bag into the open sample container. Collect the water sample only from the hot supply by only turning on the hot tap. Open the hot water tap to capture the first water coming from the showerhead. If it is a mixer tap ensure that the mixer is set to the hottest setting before opening the tap.
- **"Second Catch" sample:** (note: this sample is not necessary if a "Second Catch" sample has been taken from a simple tap for the hot water heater feeding this shower).

  This sample is taken to obtain a sample representative of the pipe work and hot water cylinder rather than colonisation of the outlet. Allow the water to run continuously for at least 2 minutes then collect a further 1 litre water sample as for "First Catch" sample, above.
- **Temperature measurement:** Take the temperature of the water at the outlet after the samples have been taken and after the water has flowed for at least 2 minutes. Disinfect the thermometer after each use with an alcohol wipe. Change gloves before taking the next sample.

A.3.5 Tap outlet (faucet)

- **Inspection:** Inspect the tap outlet noting its type, whether it is a mixer type or a direct feed from the hot or cold supply, whether it has any faults including dripping, scale build-up, or slime growth in or deterioration of the hose. Record the presence of any tempering valves or mixers
- **"First Catch" water sample:** Label a 1-Litre sample container with "First Catch". Indicate the site and that it is the 'closest to the water heater' (If there is more than one water heater then record which heater it is). Remove the cap from the "First Catch" sample container, storing it in a clean new plastic bag. Do not allow any water to run to waste. While holding the sample container under the tap, turn on

the tap to collect the first 1 litre of water and replace the cap. Shake the sample container to help dissolve the neutraliser.

- **"Second Catch" water sample:** Label another 1-Litre sample container with "Second Catch". Indicate the site and that it is the closest to the heater. Open the tap fully and let the water run to waste continuously for at least two minutes, then collect a further 1 litre of water into the "Second Catch" sample container. Replace the cap and then shake the sample container to help dissolve the neutraliser.
- **Temperature measurement:** Take the temperature of the water at the outlet after the samples have been taken and after the water has flowed for at least 2 minutes. Disinfect the thermometer after each use with an alcohol wipe. Change gloves before the next sample.

A.3.6 Hot water storage heaters (Hot water tanks)

- **Inspection:** Record if the water heater has any defects (dripping, leaking glands, etc.). Note where the outlet is and if there is a tempering valve fitted. Note if there has been any recent maintenance or if the water heater is frequently turned off.
- **Sampling:** Collect a single 1-litre water sample from each hot water storage heater used by the case. These represent "Second Catch" samples. It is generally not possible to access water heaters directly. Where direct sampling is not possible collect a "Second Catch" sample from the hot water outlet nearest to the water heater. It is preferable to collect "Second Catch" samples representative of the water in a storage tank from a simple tap rather than a mixer tap or shower to avoid mixing different water streams in the sample. But if the outlet closest to the water heater is a shower, then follow the procedures below for collecting a water sample from a shower (A.3.4). If it is a single tap, then follow the procedures below for a tap outlet (A.3.5).
- Temperature measurement: Take the temperature of the water at the outlet after the samples have been taken and after the water has flowed for at least 2 minutes. Record the thermostat setting as well. Disinfect the thermometer after each use with an alcohol wipe. Change gloves before the next sample.

A.3.7 Header tanks and cisterns

In order not to disturb sediment or biofilm prior to taking samples in the rest of the system, samples from header tanks and cisterns should be taken last.

- **Inspection:** Inspect the header tank. Note how well the tank is insulated, whether it is covered, materials of construction and the volume of water it holds.
- **Bulk water sample:** Take a 1-litre sample from the tank using the tap outlet attached to the base of the tank. Disinfect the outside of the tap with the hypochlorite solution. Disinfect the inside of the tap by squirting the hypochlorite solution into the outlet. Leave for three minutes, and then turn on the tap and allow water to run to waste for a minute before collecting any sample, and then collect a 1-litre sample without altering the flow of water. Label the sample container.
- If there is no direct outlet from the tank, collect a sample from the tank using a sterile dip sampler or by dipping the sample container into the water. If direct dipping is used then wear new disposable gloves each time. Disinfect gloved hands by washing with alcohol and allow sufficient time for residual alcohol to evaporate. Disinfect the outside of the sample container with an alcohol wipe and allow the alcohol to evaporate away before collecting the sample. Sit sample container on a clean food-grade plastic bag while waiting for the residual alcohol to evaporate. Seal the sample container and dry the outside of the sample container with a fresh paper towel.
- **Swab (biofilm) sample:** Collect a swab sample of any biofilm below the normal water line from the interior walls of the tank after the water sample has been collected. If disposable latex gloves are worn, then sterile cotton gauze (as used for wound dressings) can be used to collect the biofilm sample.
- **Temperature measurement:** Record the temperature of the water in the tank. Disinfect the thermometer after each use with an alcohol wipe. Change gloves before collecting the next sample.

A.3.8 Incoming mains water sample (or source water)

Water samples of the source water are generally not required unless it is from a non-potable water source, or if there is no active chlorine residual and the ambient water temperature is above 20°C. Also, if recent plumbing work has been carried out on water pipes to the property, then a sample of the source water should be taken.

- **Inspection:** Inspect the supply, if possible. If this is a storage tank, then note how well the tank is insulated, and whether lids are on inspection covers to minimise environmental contamination. Note the materials of construction (i.e., whether it is steel, or concrete or timber-lined) as these can influence biofilm and sediment accumulation. Also note the volume of water the tank holds. Check the amount of sediment in the tank and if any suspended matter is visible.
- **Bulk water sample:** Collect a 2 to 10-litre sample representative of the incoming water supply. Ideally this should be down stream of any storage tank(s). The bulk water sample could either be from mains water, or well water or a rain water storage tank. If collecting from a tank, either use the tap outlet attached to the base of the tank, or collect the sample from the cold water outlet closest to the water tank. Disinfect the outside of the tap with the hypochlorite solution. Disinfect the inside of the tap by squirting the hypochlorite solution into the outlet. Leave for three minutes, and then turn on the tap and allow the water to run to waste for a minute before collecting any sample, and then collect a 1-litre sample without altering the flow of water. Label the sample container.
- **Temperature measurement:** Record the temperature of the water either in the tank or at the tap where the sample is collected. Disinfect the thermometer after each use with an alcohol wipe. Change gloves before the next sample.

B. SAMPLING FROM SPA POOLS

B.1 Physical inspection

Inspect any records maintained by the pool owner to establish history and frequency of cleaning, disinfection and water changing or top-up. Record the cleanliness of the pool and its immediate environment. Note the type of biocide used. Measure and record the level of free available chlorine in the pool water if a halogen biocide is used. If a sand filter is fitted, check how often it is back-flushed.

B.2 Collection of water sample:

- Remove the sample container lid only when ready to sample. Do not insert fingers inside the sample container or the lid. Do not flush the sample container or the lid prior to sampling.
- Collect a 1-litre water sample directly from the pool using a sterile dip sampler or by dipping the sample container into the water. If direct dipping is used to collect the sample, then wear new disposable gloves each time a sample is collected. Disinfect gloved hands by washing with alcohol and allow sufficient time for residual alcohol to evaporate. Disinfect the outside of the sample container with an alcohol wipe and allow the alcohol to evaporate away before collecting the sample. Sit sample container on a clean food-grade plastic bag while waiting for the residual alcohol to evaporate. Open the sample container and place the lid inside the plastic bag. Collect the sample, then recap and shake the sample container and dry the outside of the sample container with a fresh paper towel.
- Collect a 1-litre water sample from above the filter bed.
- If a balance tank is fitted, then collect a 1-litre sample from there as well.
- Label each sample container with the location from which it was collected.

B.3 Swab (biofilm) sample collection

- Collect biofilm swab samples from both air and water inlets in the pool. The water level of the pool may need to be lowered to below the inlets before swab samples can be taken. If disposable latex gloves are worn, then a sterile cotton gauze swab (as used for wound dressings) can be used to collect the biofilm samples.
- Collect biofilm swab samples from below the normal water line of the interior surface of the pool.
- Collect biofilm swab samples from the internal pipe surfaces.
- Label each biofilm swab sample with the location from where it was collected.
- Ensure that the swab sample will not dry out by including 1-2 mL of water from the pool with each swab.

C. SAMPLING FROM COOLING TOWERS AND EVAPORATIVE CONDENSERS

Sampling and testing is based on the requirements of the AS/NZS 3666:2000 and AS/NZS 3896:1998

In accordance with Standard AS/NZS 3666.3:2000, avoid sampling for at least 72 hours after on-line disinfection or system decontamination or cleaning. This is to allow conditions to stabilise prior to sampling. Where practicable, prior to sampling, the water shall be circulated throughout the system for not less than 30 minutes. Ideally, sampling should occur while the system is in operation. The sample shall be collected from the cooling tower or a sampling point located in the cooling water return line to the cooling tower. Take the sample for *Legionella* (or other microbiological examination) before any sample required for chemical analysis to help prevent contamination of the sampling point.

C.1 Safety considerations

When collecting samples from cooling towers or evaporative condensers, wear a full-face mask fitted with a HEPA filter and eye protection. Wear rubber gloves when placing hands in water. This is to minimise exposure to both chemical and biological hazards. Do not enter a cooling tower area if it is operating without wearing appropriate safety equipment.

C.2 Physical inspection of a cooling tower or evaporative condenser

Check the water basin for an excessive build-up of sludge and organic matter. This should not be present. Check the fill material does not have an excessive scale or algae or slime. Check that drift eliminators are clean and free of debris. Check automatic dosing equipment is operating correctly

C.3 Steps for collecting water samples from a cooling tower or evaporative condenser

- Remove the sample container lid only when ready to sample. Do not insert fingers inside the sample container or the lid. Do not flush the sample container or the lid prior to sampling.
- *Collect at least 250 mL water sample:*
  - When collecting from a sampling point connected to the return line to the tower basin, allow water to run to waste for sufficient time to remove all stagnant hold-up water in the line prior to filling the sample container.
  - From a water basin, hold sample container from its base and plunge it, neck downwards, to about 50 mm below the water surface and clear of any surfaces of the cooling tower and any associated equipment. Collection of the sample shall not be taken from the vicinity of the make-up water (inlet) or near the application of any water treatment system. Turn the sample container until the neck points slightly upwards. Create an artificial current by pushing the sample container horizontally forwards and away from the hand. Avoid the collection of sludge and slime. Leave some air space in the sample container.
- Replace the cap on the sample container immediately after collection and clearly label.

C.4 Steps for collecting swab samples from a cooling tower or evaporative condenser

- Collect biofilm swab samples from the fill material and from the basin wall. If disposable latex gloves are worn, then a sterile cotton gauze swab (as used for wound dressings) can be used to collect the biofilm samples.
- Collect biofilm swab samples from any wet surface of the bottom basin.
- Collect biofilm swab samples from the surface of the fill material (cooling towers only).
- Ensure that the swab sample will not dry out by including 1-2 mL of water from the pool with each swab.
- Label each sample with the location from where it was collected.

D. SAMPLING COMPOSTED VEGETATIVE MATTER, GARDEN MULCH AND SOIL

Legionella bacteria are frequently isolated from both commercially-prepared and domestic (home-made) compost, as well as other compost-containing matrices such as potting mixes and garden mulch. Legionellae are able to persist for many months in soil and compost material, so sampling is still recommended a number of weeks after the suspected exposure event.

D.1 Physical Inspection

- If material is home-made, record as such on sample submission form. If a commercial product, record name of manufacturer, name of product, and date and place it was purchased. Also inspect container for evidence of any health warnings or other health information.

D.2 Steps for collecting composted material

- Put on a correctly fitting N95 or N99 face respirator covering both mouth and nose before opening any bags or bins containing composted material. Check the condition of the material. When collecting samples from bags that have been left opened ensure the sample is collected only from the 'dampest' material.
- Thoroughly mix the suitable material to ensure a representative sample is obtained. If the material cannot be mixed, then take samples from a number of different points and combine to obtain a representative sample.
- The sample container should remain unopened until just prior to sampling. Using sterile sampling equipment, e.g. scoop, spoon etc., transfer approximately 100 grams into a sterile sample container. A freshly disinfected scoop, etc. must be used for each subsequent sample.
- A minimum of 100 grams of material is needed for a valid sample size. Seal the sample container to prevent the material drying out. Clearly label the sample.

If a sterile scoop is not available, an alternative method to obtain a sample is as follows:

- Put on fresh latex gloves and wash the outside of the gloves with an alcohol disinfectant. Allow gloves to air-dry to remove excess disinfectant then with gloved hands turn a zip-lock plastic bag inside out and place the bag over a gloved hand. Using the hand inside the bag, take a large handful of compost material and then with your free hand pull the bag back off your fist to capture the material in the bag.
- A minimum of 100 grams of material is needed for a valid sample size. Seal the sample container to prevent the material drying out. Clearly label the sample.

If no material is left for sampling from bags or bins, then the next best alternative site for sampling is the soil (garden or pot) where the compost material has been incorporated.

- Take a representative sample from two to three sites, each at least 10 cm below the surface. This avoids obtaining material exposed to excessive sunlight or moisture loss.
- A minimum of 100 grams of material is needed for a valid sample size. Seal the sample container to prevent the material drying out. Clearly label the sample.

E. NATURAL POOLS, GEOTHERMAL BATHS & DECORATIVE FOUNTAINS

E.1 Physical Inspection

- Inspect the water feature for a build up of organic matter, slime algae or debris. Check the type of material forming the lining of the water feature i.e., whether it is clay or earth or wood, or grouted tiles or a mixture of these. Check for the presence of spaying or misting devices - if any are located, swab-samples need to be collected from these.
- Check if there is any chemical or physical treatment process for the water feature. Measure the Free Available Chlorine levels at the time water samples are collected.
- Measure the temperature of the water with a graduated and calibrated thermometer.

E.2 Water sample collection

- Remove the sample container lid only when ready to sample. Do not insert fingers inside the container or the lid. Do not flush the container or the lid prior to sampling.
- Collect at least a 1-litre sample of water from the pool.
- From a sampling point, allow water to run freely for a minimum of 30 seconds prior to collecting a 1-litre sample.

E.3 Swab sample collection

- Collect swab samples from any wet surface where biofilm can accumulate.

- Collect swab samples from any nozzle or spray outlet - these may require some disassembly to access the interior surfaces.

CONSIDERATION OF OTHER POTENTIAL SOURCES OF LEGIONELLA CONTAMINATION

In the investigation of any CAP case of legionellosis, consider the sites listed below as potential sources for *Legionella*. For specific sampling instructions, please contact the EML for advice. Generally, collect at least one litre of water from any potable (drinking water) source and 250-mL from any raw water source. Swab samples should be taken from any surface where biofilms can accumulate. Sediment samples can be collected for Legionella culture testing from the bottom of water storage tanks.

The sources listed below have been the implicated in either sporadic legionellosis cases or outbreaks and should also be sampled:

- Air washers (scrubbers)
- Dental equipment (e.g., water cooled high speed drills)
- Drinking water from a water cooler or bottle reservoir
- Filter equipment from in-line drinking water filtration systems
- High pressure water sprayers
- Humidifiers and nebulising equipment
- Ice machines
- Misting machines (e.g, vegetable and fruit misters)
- Vehicle washers including car wash facilities
- Water-cooled machine tools – especially where aerosols are generated

When inspecting any likely water source, consider the following:

- Is the water untreated or is there no active chlorine residual?
- Is it able to reach temperatures above 20°C but below 55°C?
- Is there any external heat source near any part of the water distribution system that can warm the water?
- Is there any means of aerosolising the water?
- Is the water recirculated or stored for long periods?
- Is there any build-up of sludge or organic material?
- Has the water
- Has any major plumbing work recently happened at the property
- Has part of a recirculating system been recently cleaned by the case?

If the answers to any of these questions are ‘yes’, then sampling for Legionella bacteria should be considered.

SITES NOT CONSIDERED POTENTIAL SOURCES FOR *LEGIONELLA*

The sites listed below have been suspected as likely sources for *Legionella* infection, but to date have not been proven as an infection source for a case:

- Instant water heaters (both gas and electrical) where there is no water storage reservoir
- Evaporative coolers (air chillers and heat pumps)
- Dehumidifiers
- Vehicle air conditioning units

SAMPLE PACKING AND SHIPPING

Ensure each sample is labelled with the following:

- the type of sample
- the sample site
- the sampling date
- a unique sample identifier
- who collected the sample

Include a completed sample submission form with a list of the samples and the details of each sample itemising the sites from where they were collected, the date shipped and the case's EpiSurv #. The sample submission form is available from the ESR website at http://www.esr.cri.nz/assets/Forms/ESR04-Legionella-sample-request-form.pdf

Inform the EML of the shipment of any samples and their courier tracking number prior to sending.

Do not send samples overnight on a Friday, as samples cannot be receipted on a Saturday.

Ensure all sample containers are securely sealed to avoid leakage and contamination. Do not ship any samples in a glass container.

Water and swab samples must be packed into a container that protects the samples from exposure to light and temperature fluctuation. A chilli bin is an ideal container for this. Do not pack any samples with chilled or frozen ice packs or chiller packs.

All samples other than compost material must be sent immediately after collection to the Environmental Microbiology Laboratory by courier. Do not send water or swab samples by post. It is acceptable to send compost material by post as long as it is securely packaged and sealed as this material is more stable than water.

All samples other than compost material must reach the laboratory within 24 hours of collection. Compost material can take up to three days to reach the laboratory.

Compost samples must be sent protected from the light in a sealed container.

Testing of water samples takes a minimum of ten working days with results reported after that time. Compost samples can take up to 20 days to before a final result is available

CONTACT DETAILS

Mark packages: For Attention of: Environmental Microbiology Laboratory

Forward samples by courier to:

Street Address: Environmental Microbiology Laboratory
ESR Ltd
Kenepuru Science Centre
34 Kenepuru Drive
PORIRUA 5022

Postal address: P O Box 50-348
Porirua 5240

Contact details: Tel: 04 914 0755 or 04 9140697
Fax: 04 9140770
Email: KSC.Legionella@esr.cri.nz