

**Legionnaires' disease:
NICD Recommendations for
Diagnosis, Management
and
Public Health Response**

**Compiled by the Centre for Respiratory Diseases and Meningitis,
National Institute for Communicable Diseases (NICD) of the
National Health Laboratory Service (NHLS)**

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Disclaimer:

The information contained in this document, be it guidelines, recommendations, diagnostic algorithms or treatment regimens, are offered in this document in the public interest. To the best of the knowledge of the guideline writing team, the information contained in these guidelines is correct. Implementation of any aspect of these guidelines remains the responsibility of the implementing agency in so far as public health liability resides, or the responsibility of the individual clinician in the case of diagnosis or treatment.

Quick Reference Guide – Legionellosis

Legionellosis case definitions: Page 8

A confirmed case of Legionnaires' disease:

Any person with clinical/radiological evidence of pneumonia **AND** isolation of *Legionella* spp. from a clinical specimen, detection of *L. pneumophila* serogroup 1 antigen in urine, or *L. pneumophila* serogroup 1 specific antibody response.

A probable case of Legionnaires' disease:

Any person with clinical/radiological evidence of pneumonia **AND** detection of *Legionella* spp. nucleic acid in a clinical specimen, or *L. pneumophila* non-serogroup 1 or other *Legionella* spp. specific antibody response.

Treatment of Legionnaire's disease Page 9

- Patients with Legionnaires' disease require early treatment with a macrolide or fluoroquinolone antibiotic.
- Recommended duration for antimicrobial therapy is 7 to 10 days, and up to 21 days for immunosuppressed patients.
- Beta-lactam antibiotics are not effective.

Notification of cases and additional support:

Laboratory support: National Institute for Communicable Diseases, Centre for Respiratory Diseases and Meningitis: Nicole Wolter 011-555-0352 nicolew@nicd.ac.za, or after-hours, the NICD doctor-on-call 082 883 9920:

Public health support and notification of cases: Notify the Provincial Communicable Diseases Control Officer, or the NICD Outbreak Response unit (011-555-0542) or outbreak@nicd.ac.za.

Diagnosis of Legionnaire's disease: Page 7

For a patient with suspected Legionnaires' disease the following specimens should be collected: Urine and sputum or other lower respiratory tract specimen (bronchoalveolar lavage, tracheal aspirate, pleural fluid or lung tissue).

Diagnostic tests include:

1. Urinary antigen test (urine specimen) – detects *L. pneumophila* serogroup 1
2. Culture (sputum or respiratory specimen) – detects all *Legionella* spp.
3. Polymerase chain reaction (sputum or respiratory specimen) – detects all *Legionella* spp.

Public health response to Legionnaire's disease Page 9

Legionellosis is a notifiable condition. If a confirmed **OR** probable case is detected:

1. The clinician must notify the District CDC, and complete Form GW 17/5
2. The District CDC must investigate the case through completion of a case-investigation form (CIF).
3. The District CDC should inform the NICD and forward all available documentation
4. The diagnostic laboratory or microbiologist should inform the attending clinician and NICD and submit specimens and isolates to NICD.

If a cluster (≥ 2) of cases of Legionnaires' disease with epidemiological links is identified during a 12 month period, an outbreak investigation and environmental assessment will be conducted.

Environmental assessment for *Legionella* spp.

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- An environmental assessment includes environmental sample collection for laboratory testing.
- Emergency and long-term remediation measures may be recommended.
- Continuous, thorough and routine maintenance and treatment is required to prevent growth of the *Legionella* bacteria.

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1. Introduction

Legionellosis, or disease caused by bacteria from the genus *Legionella* is a notifiable condition in South Africa. Infection is acquired from inhalation of contaminated aerosols. Infection with *Legionella* commonly may present with a spectrum of illness ranging from asymptomatic, to severe pneumonia (Legionnaire's Disease (LD)), often requiring hospitalization. The disease has a case-fatality ratio of 10-15%¹.

These guidelines have been drawn up to assist with the diagnosis, management and public health response to Legionnaires' disease in South Africa.

2. Microbiology

Legionnaires' disease is caused by the gram-negative bacterium *Legionella*. More than 50 species of *Legionella* have been described, however only approximately 20 have been associated with disease in humans¹. *Legionella pneumophila* serogroup 1 accounts for the majority of clinical cases, causing up to 90% of laboratory-diagnosed cases in the US and Europe^{1,2}. Whereas in other parts of the world, such as Australia, *L. longbeachae* (found in compost and potting soil) is predominant. Data on the prevalence of *Legionella* species are limited in South Africa. *Legionella* bacteria are ubiquitous and exist in natural water sources such as lakes and streams, although transmission is predominantly associated with warm man-made water systems which provide the 3 conditions needed for transmission: heat (20°C to 45°C), stasis and aerosolisation. Potential sources of infection include³:

- Hot and cold water systems
- Cooling towers and evaporative condensers
- Spa pools / natural pools / thermal springs
- Fountains / sprinklers
- Respiratory therapy equipment
- Potting soil / compost
- Car washes
- Water-cooled machine tools

3. Epidemiology of Legionnaires' disease

Legionnaires' disease may present in three epidemiological scenarios³: 1) as an outbreak of 2 or more cases following a spatial and temporal exposure to a single source, 2) as a series of independent cases in an area in which it is highly endemic, or, 3) as sporadic cases without any obvious temporal or geographical grouping. The majority of cases of LD are isolated and sporadic. Illness can occur any time of the year, however it occurs more commonly in the summer and early autumn seasons.

LD may be community-acquired, however it is more commonly associated with nosocomial transmission (hospital-associated LD) and travel. Immunocompromised patients in health-care settings are at increased risk of developing Legionnaires' disease if exposed to contaminated water, whereas the complex water systems of large buildings are more prone to *Legionella* contamination.

Globally, *Legionella* spp. account for 2-5% of community-acquired pneumonia (CAP) cases in adults and are rarely detected in children^{1,2}. However, Legionnaires' disease is considerably underdiagnosed and underreported. It is estimated that less than 5% of cases are reported to public health authorities through passive surveillance. *Legionella* spp. are more commonly associated with

sporadic disease, however may cause outbreaks. In the United States (US), between 8,000 and 18,000 people are hospitalized with Legionnaires' disease each year. This, however, is likely to be an underestimate as most infections are not diagnosed or reported⁴.

Data on the epidemiology of Legionnaires' disease in South Africa are limited. In a recent study of syndromic pneumonia surveillance at two sentinel sites in South Africa from June 2012 through September 2014, *Legionella* spp. were detected in 21 (1.2%) of 1805 cases. This study reported that community-acquired LD in South Africa occurs predominantly in chronically ill adults with HIV and/or TB infection, and the majority of cases are not diagnosed and are sub-optimally treated⁵. Hospital-acquired and travel-associated cases of Legionnaires' disease have been reported in South Africa. As in other parts of the world, the prevalence of Legionnaires' disease in South Africa is underestimated due to a lack of clinical index of suspicion and request for testing by clinicians who generally treat empirically for CAP, inadequate diagnostic tests and limited surveillance programs¹.

Legionnaires' disease may be classified into the following three categories based on the source of exposure⁶:

Travel-associated case: a case that has a history of spending at least one night away from home, either in the same or different country, in the two weeks before onset of illness

Nosocomial case: a case that stayed or spent time (e.g. as an outpatient) in a hospital or healthcare facility in the two weeks before onset of illness

Community-acquired case: a case with no history of overnight stays outside of the home or hospital admission or association with a healthcare facility in the two weeks before onset of illness

4. Pathogenesis, pathology and transmission

Legionella pneumophila is a facultative intracellular bacterium that can invade human macrophages and can also replicate inside amoebae, which can serve as a reservoir for *L. pneumophila*, as well as provide protection from environmental stresses, such as chlorination^{1,7}.

Legionnaires' disease is usually acquired through the respiratory system by the inhalation of air droplets that contain *Legionella* bacteria. An aerosol is formed from tiny droplets that can be generated by spraying the water or bubbling air into it. More rarely, aspiration of contaminated water has been the cause of disease. Human-to human transmission is not common, and only one probable case has recently been reported⁸. After inhalation, symptoms usually commence within 2 to 10 days, but may commence up to 3 weeks after exposure.

Although *Legionella* bacteria are ubiquitous in the environment, they rarely cause disease. A combination of factors are required for disease to develop: (i) presence of a virulent strain in a water source (or soil in the case of *L. longbeachae*), (ii) means for dissemination (aerosolisation) of the bacteria, (iii) environmental conditions allowing the survival an inhalation of an infectious dose of the bacteria, (iv) a susceptible host^{1,2}. Once the bacteria enter the lung, they are phagocytosed by alveolar macrophages, multiply within the macrophage which leads to death of the macrophage and releases large numbers of bacteria into the extracellular environment. These bacteria are then re-phagocytosed by macrophages, resulting in intracellular multiplication of the bacteria within the alveoli of the lung^{1,7}.

5. Clinical presentation and risk factors

Host risk factors for LD include those that result in decreased local or systemic cellular immunity and those that increase the chances of exposure to an infectious aerosol or microaspiration of contaminated water¹. Recognised personal risk factors include the following: Older age (≥50 years), male gender, chronic underlying disease including diabetes and heart or lung disease, HIV and TB, high alcohol intake, current or past history of heavy smoking, immunosuppression / immune system disorders such as organ transplant recipients or persons receiving chemotherapy.

Environmental risk factors include activities that increase the chances of exposure to contaminated water include recent overnight travel, use of well water in the home, recent plumbing work within the home, disruptions of water supply resulting in “brown” water at the tap, using an electric water heater, use of or proximity to a spa pool, living in close proximity to a cooling tower, or being near decorative fountains^{1,7}. In addition, nosocomial exposures include delivery of *Legionella*-contaminated water (through tap water filled or rinsed nebulizers, humidifiers, ventilator tubing, nasogastric feedings or lavages) into the respiratory tract.

Legionellosis is associated with two clinically and epidemiologically distinct illnesses; Legionnaires’ disease (LD) and Pontiac fever. Legionnaires’ disease is a relatively uncommon form of pneumonia, which has a high case-fatality rate of 10-15% (up to 30%). Symptoms include flu-like illness (high fever, muscle aches, headaches), followed by a dry cough and progression to pneumonia⁷. Approximately 20-50% of people with LD may also present with diarrhoea, and approximately 50% may show signs of mental confusion. If not treated, the symptoms normally worsen rapidly and may result in respiratory failure, shock, multi-organ failure and death. Situations suggesting LD include^{1,7}: 1) Gram’s stains of respiratory samples revealing many polymorphonuclear leukocytes with few or no organisms; 2) the presence of hyponatremia, 3) pneumonia with prominent extrapulmonary manifestations (eg. diarrhoea, confusion, other neurologic symptoms), 4) failure to respond to administration of beta-lactams, aminoglycoside antibiotics, or both.

Pontiac fever is a non-pneumonic illness also caused by *Legionella* bacteria^{1,7}. It has a shorter incubation period of 12-48 hours, presents as a mild flu-like illness, and lasts up to a few days. The illness is self-limiting, and no antibiotic treatment is necessary for this illness.

6. Laboratory diagnosis of Legionnaires’ disease

Legionnaires’ disease presents with an acute consolidating pneumonia, which can be radiologically and clinically indistinguishable from other aetiological causes of pneumonia^{1,7}. Therefore laboratory investigations must be carried out to obtain a diagnosis. The following patients should be tested for LD⁴: 1) Patients with pneumonia who have failed empiric antibiotic therapy; 2) Patients with severe pneumonia, in particular those requiring intensive care; 3) Immunocompromised individuals with pneumonia; 4) Patients with pneumonia in the setting of a legionellosis outbreak; 5) Patients who have travelled away from their home within two weeks before the onset of illness; 6) Patients suspected of health-care associated pneumonia.

6.1 Specimen collection

For a patient with suspected Legionnaires’ disease the following specimens should be collected:

- Urine specimen for antigen testing

- Sputum specimen (as this disease presents with a dry cough, the sputum may need to be induced), or
- Other respiratory samples such as, bronchoalveolar lavage, tracheal aspirates, pleural fluid or lung tissue (trans-bronchial biopsy) for detection of the organism by culture or PCR.

A nasopharyngeal specimen (in transport medium such as Cary Blair, Universal transport medium, or Primestore molecular transport medium) may be collected if a sputum specimen cannot be obtained, although this is not recommended. If the NP specimen tests positive for *Legionella* spp., the result will confirm the diagnosis however, due to the low sensitivity of the specimen type a negative test result does not exclude *Legionella* infection. Oropharyngeal swabs are not recommended for the diagnosis of LD.

Urine and respiratory specimens should be collected in a sterile container. Specimens should be immediately refrigerated at 2-8°C after collection and transported to the laboratory on ice or ice-packs in a cooler box. If possible for lower respiratory tract specimens, freeze the specimens after collection and transport to the laboratory on dry-ice.

6.2 Diagnostic tests and specimen types

Table 1 lists diagnostic tests for LD, and the appropriate specimen types on which they should be performed^{1,7}. The most commonly used diagnostic test for LD is the detection of *Legionella* antigen in a urine specimen during the acute phase of illness. The urinary antigen test (UAT – *in vitro* rapid immunochromatographic assay) is rapid and inexpensive, although it only detects the most common strain of *Legionella*, *Legionella pneumophila* serogroup 1. As the urinary antigen test only detects *Legionella pneumophila* serogroup 1, a negative test does not exclude Legionnaires' disease.

The “gold standard” diagnostic method remains culture from a respiratory specimen, which enables strain characterisation¹. Culture is an important test as it allows for comparison of strains from environmental and clinical sources, as well as the identification of less common strains⁴. Investigations of outbreaks of Legionnaires' disease rely on a comparison of environmental and clinical isolates. For culture, the specimen should be cultured on buffered charcoal yeast extract (BCYE) agar containing 0.1% α-ketoglutarate with L-cysteine and incubated at 35°C in a humidified (sealed plastic bag), 2.5% CO₂ atmosphere. Most isolates grow within 3-5 days. However, a negative result is only released after 7-10 days of incubation.

More recently, real-time PCR on respiratory specimens is also used. PCR is able to detect all species of *Legionella*. However the disadvantage is that no culture isolate is available for comparison with environmental strains.

Table 1. Diagnostic tests and specimen types for the diagnosis of Legionnaire’s disease

Test	Specimen	Species identified	NHLS and private laboratories offering testing
Urinary antigen	Urine	<i>Legionella pneumophila</i> serogroup 1	<ul style="list-style-type: none"> NHLS Groote Schuur CRDM, NICD NHLS Infection Control Lab Some private laboratories
Culture	Sputum /Other lower respiratory tract samples	<i>Legionella pneumophila</i> serogroup 1 <i>Legionella pneumophila</i> serogroups 2-14 <i>Legionella</i> spp.	<ul style="list-style-type: none"> NHLS Infection Control Lab CRDM, NICD
PCR	Sputum / Other lower respiratory tract samples	<i>Legionella</i> spp. <i>Legionella pneumophila</i> serogroup 1 <i>Legionella longbeachae</i>	<ul style="list-style-type: none"> CRDM, NICD Some private laboratories

7. Case definitions

In order to appropriately investigate a case, or outbreak of LD, the following case definitions are proposed for the South African setting. Clinical and laboratory criteria are listed in Table 1^{3;4;6}.

A probable case of LD: Any person meeting the clinical criteria AND at least one laboratory criteria for a probable case

A confirmed case of LD: Any person meeting the clinical criteria AND at least one laboratory criteria for a confirmed case

Table 1. Clinical and laboratory criteria for the diagnosis of Legionnaire’s disease.

Clinical criteria for the diagnosis of LD:	Clinical or radiological evidence of pneumonia
Laboratory criteria for the diagnosis of LD:	
A <u>confirmed case</u> of LD requires at least one of the following:	Isolation of <i>Legionella</i> spp. from a respiratory specimen or any normally sterile site Detection of <i>Legionella pneumophila</i> serogroup 1 antigen in urine <i>Legionella pneumophila</i> serogroup 1 specific antibody response (fourfold or greater rise in specific serum antibody titer)
A <u>probable case</u> of LD requires at least one of the following:	Detection of <i>Legionella</i> spp. nucleic acid in a clinical specimen <i>Legionella pneumophila</i> non-serogroup 1 or other <i>Legionella</i> spp. specific antibody response (fourfold or greater rise in specific serum antibody titer)

8. Treatment of Legionnaire's disease

According to the South African guidelines for community-acquired pneumonia (CAP), empiric antibiotic therapy for CAP patients is amoxicillin, which should be replaced with co-amoxiclav if there is structural lung disease or recent antibiotic use⁹. A macrolide (usually azithromycin) should be added to beta-lactam therapy in patients with severe CAP. *Legionella* infections, which are intracellular pathogens, do not respond to β -lactam antibiotics like penicillins and cephalosporins and therefore will not be covered by empiric therapy for CAP in South Africa. Patients with Legionnaires' disease require early treatment from an appropriate range of antibiotics which can penetrate the cells such as macrolide or fluoroquinolone antibiotics^{1,7}. Therefore it is important for clinician's to have a high index of suspicion for *Legionella* infection and request appropriate diagnostic tests. Recommended duration for antimicrobial therapy is 7 to 10 days but some authors recommend up to 21 days for immunosuppressed patients.

9. Prevention of Legionnaire's disease

The proper design, maintenance and temperature of potable water systems are the most important method for preventing the amplification of *Legionella*⁷. Hot water should be stored above 60°C and delivered to taps above 50°C. Cold water should be stored below 20°C, and dead legs or low flow areas eliminated. There are currently no vaccines available for the prevention of Legionnaires' disease, and prior infection does not necessarily prevent reinfection¹.

10. Public health response to Legionnaires' disease

The majority of Legionnaires' disease cases are isolated and sporadic. Outbreaks are commonly associated with buildings or structures that have complex water systems, such as hotels, hospitals and cruise ships. The most likely sources of infection include water used for drinking and showering and air-conditioning cooling towers.

Prompt notification of public health authorities of any suspected or confirmed case is critically important for detecting epidemics of the disease. Legionnaires' disease is a notifiable condition in South Africa.

10.1. Response to a single case of Legionnaires' disease

If a confirmed or probable case is detected the following steps should be taken at least within 3 days of diagnosis:

1. The clinician must notify the District Communicable Diseases Co-ordinator (CDC), and complete Form GW 17/5
2. The District CDC must investigate the case through completion of a case-investigation form (CIF). This will require an interview with the patient or close relative to identify potential sources of infection. The CIF can be found on the NICD web-site.
3. The District CDC should inform the NICD (Dr Kerrigan McCarthy: kerriganm@nicd.ac.za or Dr Nicole Wolter: nicolew@nicd.ac.za) as soon possible and forward all available documentation (lab results, notification, CIF) by email.
4. The diagnostic laboratory or microbiologist should inform the attending clinician and NICD (Kerrigan McCarthy: kerriganm@nicd.ac.za or Nicole Wolter: nicolew@nicd.ac.za) and submit specimens and isolates as soon as possible to the Centre for Respiratory Diseases and Meningitis

(CRDM) bacteriology laboratory, National Institute for Communicable Diseases (NICD), 1 Modderfontein Road, Sandringham, Johannesburg, 2131.

It may not be necessary to conduct an environmental assessment when a single, isolated case of LD has been identified. However, the facility (the putative source of infection – the hotel or hospital or other institution) should be notified of the case to raise awareness of the risk of legionellosis so that preventative measures can be strengthened. All potential nosocomially-acquired cases of infection will be further investigated to confirm/exclude the hospital as the source of infection, and environmental sampling conducted when considered necessary.

Case definitions for nosocomial Legionnaires disease^{6;7}:

- Definite nosocomial- Legionnaires' disease in a person who was in hospital or other healthcare facility for at least 10 days before the onset of symptoms.
- Probable nosocomial – Legionnaires' disease in a person who stayed or spent time (eg. as an outpatient or healthcare worker) in a hospital or other healthcare facility for 1-9 of the 10 days before the onset of symptoms, and either became ill in a hospital associated with one or more previous cases of Legionnaires' disease, or yielded an isolate that was indistinguishable from the hospital water system at about the same time.
- Possible nosocomial – Legionnaires' disease in a person who was stayed or spent time (eg. as an outpatient or healthcare worker) in a hospital or other healthcare facility for 1-9 of the 10 days before the onset of symptoms, in a hospital not previously known to be associated with any case of legionnaires' disease, and where no microbiological link has been established between the infection and the hospital.

10.1 Response to a cluster of cases of Legionnaires' disease

If a cluster (≥ 2) of cases of Legionnaires' disease with epidemiological links to a specific location, such as a hotel or health facility, is identified during a 12-month period, it becomes necessary to conduct a full environmental assessment of the specific location. The steps listed above should be followed for each case identified. The Department of Health will request an outbreak investigation which should be initiated as soon as possible (ideally within 24 hours) after the notification of 2 or more probable/confirmed cases. This may include an environmental assessment as well as environmental sample collection for laboratory testing. The number and type of samples depend on the size and complexity of the facility, as well as the location of the reported cases. It may also be necessary to establish if additional persons who are currently or were resident at the facility have developed or are at risk for LD. A template letter in Appendix 1 may be used to inform persons of their risk.

Environmental sampling (Appendix 2) includes 1L water collection of water sources in sterile plastic bottles, as well as swabs of biofilms. At the time of collection water temperature is monitored and recorded. Samples are transported immediately to the NHLS Infection Control laboratory in Johannesburg for culture and testing. If the culture is positive, *Legionella* serogroup 1 or *Legionella* serogroups 2-14 OR *Legionella* spp. can be identified & quantified.

Based on the findings of the investigation, a final report with recommendations for remediation and control measures will be provided. Both emergency and long-term remediation measures are recommended. Emergency remediation may include heat disinfection or chemical disinfection (hyperchlorination), and should be carried out as soon as the cluster has been identified but not

before samples have been collected. Remediation should be conducted in consultation with an accredited water treatment company.

There are however no permanent solutions and ongoing maintenance of water systems is necessary. Continuous, thorough and routine maintenance and treatment is required to prevent re-growth of the *Legionella* bacteria. Further follow-up environmental sampling may be recommended.

11 Additional information:

If you require additional information, please contact the NICD: Kerrigan McCarthy 011-555-0542 kerriganm@nicd.ac.za or Nicole Wolter 011-555-0352 nicolew@nicd.ac.za, or after-hours, the NICD doctor-on-call 082 883 9920.

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13 Appendices

Appendix 1: Template letter to inform individuals of potential exposure following recognition of an outbreak of Legionnaire's disease at an identified location

[Address of sender]

[Contact details of sender]

[Date of letter]

[Address of recipient]

Dear [Name of person resident in implicated facility]

Re: *Legionella* infections in [Name of institution / hotel / facility]

Legionnaires' disease has been diagnosed in a number of individuals that have previously visited **[Name of institution / hotel / facility]**

We are writing to you because you have been resident at [Name of institution / hotel / facility] and there is a chance that you may have been infected with this disease. Legionnaire's disease is an uncommon form of pneumonia caused by a type of bacterium that is found in the environment. It causes disease when it is spread through the air as a spray or vapour from a water source and droplets are inhaled. Spread from one person to another is uncommon.

The symptoms of Legionnaires' disease include a 'flu-like' illness with muscle aches, tiredness, headaches, dry cough and fever, leading on to pneumonia. Sometimes diarrhoea occurs and patients may suffer from confusion. It can be treated with antibiotics. The period between infection and symptoms developing (the incubation period) ranges from 2 to 19 days. In rare cases some people may develop symptoms as late as three weeks after exposure.

If you experience the symptoms outlined above please contact your doctor and take a copy of this letter with you.

If you require further information please see the Frequently Asked Questions document on the NICD website (www.nicd.ac.za). Please contact us using the contact details provided below. The following international websites may be helpful

<http://www.cdc.gov/legionella/index.html>

http://ecdc.europa.eu/en/healthtopics/legionnaires_disease/Pages/basic_facts.aspx

Yours sincerely,

[Name of Sender]

[Email and telephone numbers of the Sender]

Appendix 2: Procedures for collecting water samples for *Legionella* detection in a hospital, hotel or large building

INTRODUCTION

Legionella may be found in the various water systems of a large building and the following are principles for guidance in effective sampling. Please liaise with Mr Rob Stewart (Rob.Stewart@nhls.ac.za, 011 489 8578) or Dr Teena Thomas (teena.thomas@nhls.ac.za, 011 489 9181) at the NHLS Infection Control laboratory before collecting specimens for *Legionella* testing.

PLANNING

The first task is to map the water systems in the building with the maintenance manager or a person with a working knowledge of the plumbing and air conditioning systems.

Representative samples should be taken so that the different systems in the building are all sampled. Hot and cold water systems as well as open air conditioning systems should be sampled.

The number and frequency of sampling will be affected by the budget so it is best to start with high-risk areas first.

SAMPLING

A one litre sample should be taken from each sample site.

The sample should be taken immediately as the tap is opened. An immediate sample is most representative of the colonization of the outlet and gives the best indication of risk to the user.

Samples may be transported at room temperature to the testing lab, provided they will be delivered within 48 hours. If there is a likelihood of prolonged delay in transit then the samples should be placed in a cooler box with cooler bricks.

Sampling for hotels and large buildings

Take hot & cold water samples from the following areas:

- High risk areas defined as areas where one or more *Legionella* cases have been confirmed or areas where many people are potentially exposed.
- The tap most distant in the building from the mains inlet (cold).
- Representative samples from each wing
- Representative samples from each floor
- Cold water holding tank /s (roof tank /s)
- Central hot water tank /s or representative samples from geysers
- The tap most distant from the hot water boiler unit
- Separate buildings
- Hot water taps where the temperature does not reach 50°C within 30 seconds
- Hot water taps where the flow is very slow
- Cold water taps where the temperature is too high (lukewarm)
- Taps that have thermostatic mixing valves to regulate the temperature
- Showers and taps that are seldom used may be tested in a high risk area but ideally they should be flushed on a regular basis (weekly)
- New areas of the building not utilized yet
- Any rooms or floors that have not been in use (stagnation of water)
- Decorative water features inside or outside the building

- Cooling towers from the Cooling tower pond
- Air conditioning sumps
- Jacuzzi
- Swimming pools
- Sauna
- Gym
- Ice machines
- Water stations (25 l water bottles)
- Misting devices
- Natural thermal springs and water distribution system
- Heat exchangers
- Chillers
- Pumps
- Feed tanks
- Humidifiers
- Irrigation systems
- Cistern of toilets and especially from disabled toilets

Sampling for hospitals

Take hot and cold water samples from the following areas:

- High risk areas such as: bone marrow and other transplant units, oncology & surgical unit, ICU, Renal unit, Neonatal ICU, Theatre
- The tap most distant in the building from the mains inlet (cold).
- Representative samples from each wing
- Representative samples from each floor
- Cold water holding tank (roof tank)
- Central hot water tank / representative samples from geysers
- Separate buildings
- Hot water taps where the temperature does not reach 50°C within 30 seconds
- Hot water taps where the flow is very slow
- Cold water taps where the temperature is too high (lukewarm)
- Taps that have thermostatic mixing valves to regulate the temperature
- Showers and taps that are seldom used may be tested in a high risk area but ideally they should be flushed on a regular basis (weekly)
- New areas not utilized yet
- Decorative water features inside the building
- Cooling towers
- Air conditioning sumps

TEMPERATURES

It is important to take temperatures of hot and cold water at all the sites where samples are taken. The general guideline is that cold water temperatures $\geq 20^{\circ}\text{C}$ and hot water temperatures $\leq 50^{\circ}\text{C}$ indicates conditions favourable for the amplification of *Legionella* organisms.

A thermometer that has been calibrated or validated against a calibrated thermometer should be used.

The tap temperature should be taken after the water has run for 60 seconds.
Beware of cross-contaminating water samples with the thermometer probe. Use 70% alcohol to disinfect the probe between samples.

TESTING OF ENVIRONMENTAL SAMPLES AT NHLS INFECTION CONTROL LABORATORY

Environmental samples may be sent to NHLS Infection Control Laboratory, Charlotte Maxeke Hospital, Johannesburg, for *Legionella* testing. Please contact Mr Rob Stewart (Rob.Stewart@nhls.ac.za, 011 489 8578) or Dr Teena Thomas (teena.thomas@nhls.ac.za, 011 489 9181) for more information.

Note the following guidelines:

1. The required sample size for *Legionella* analysis is one litre of water.
2. Sample bottles (new, unused plastic containers are suitable) may be obtained from the NHLS Infection Control services laboratory. Please do not use glass sample bottles as these are prone to breakage during transit.
3. Specimen bottles must be clearly labelled with marker pen. If a number is placed on the lid it must also be on the side of the sample container.
4. Please send a list of samples that have been dispatched in the box of samples. We cannot process samples without a request form (available on request).
5. If possible, samples should not be dispatched on a Friday or before a Public holiday or long weekend to avoid delays in transit. It is preferable if you send samples as early in the week as possible.
6. The best practice is to process samples as soon after they are taken as possible so please avoid delays in dispatching samples to us.
7. Samples should be kept at room temperature if they will arrive at the lab within 24 hours of being taken. If longer delays are anticipated or if the samples will be subjected to high temperatures during transit, then they must be transported in a cooler box with ice bricks.
8. It will take 10 – 14 days for your results to be ready.
9. Please supply the email address to which results must be sent to.
10. The name of the sender plus all contact details such as landline, cell number and email address are required on the request form.